Saskatchewan
Ministry of
Agriculture

FINAL REPORT

RESEARCH

ADF

AGRICULTURE DEVELOPMENT FUND

20040495

DEVELOPMENT OF A "DOUBLE LOW" FEED BARLEY FOR RUMINANTS

Funded by: The Agriculture Development Fund

November 2010

Prepared by: University of Saskatchewan (U of S)

Development (Investigation) of a "Double Low" Feed Barley for Ruminants

Investigation of a 'Double Low" Feed Barley with Low Hull Content and Low Content of Hydroxycinnamic Acids in the Hulls for Ruminants

ADF Project # 20040495

Final Report

July, 2010

By
Ministry of Agriculture Strategic Research Chair/Associate Professor
Peiqiang Yu[1*]

Graduate students
Gali (L.) Du, and Chris (N.) Liu

Project Collaborators and Graduate Student Committee Members
B.G Rossnagel[2], J.J. McKinnon[1], V.J. Racz[3], and D.A. Christensen[1]

[1] Department of Animal and Poultry Science, University of Saskatchewan
[2] Crop Development Centre, Department of Plant Sciences, University of Saskatchewan
[3] Feed Innovation Institute, University of Saskatchewan

[*]Corresponding Author and Graduate Students' Supervisor

Table of Contents

Abstract 8
Executive Summary 10
Research Progress 11
Introduction 11
Overall Project Objectives 11
Completed Projects 12
PART I
Project 1: INVESTIGATION OF CHEMICAL AND PHYSICAL DIFFERENCES AMONG THE VARIOUS BARLEY VARIETIES GROWN IN WESTERN CANADA 12
Project Details 12
Introduction 12
Objective 13
Materials and Methods 13
Barley Varieties and Samples 13
Determination of Barley Hull Content Using Modified European Brewery Convention (EBC) Method 14
Determination of the Content of NDF, ADF and ADL 14
Determination of Ferulic Acid and ρ-Coumaric Acid Content 14
Determination of Mean/Median Particle Size of Barley Grain Obtained after Dry-rolling 15
Statistical Analysis 16
Results and Discussion 16
Variety Effect on Barley Hull Content 16
Variety Effect on the Content of FA, PCA and PCA/FA in Barley Grain 17
NDF, ADF, ADL, Hemicellulose and Cellulose in Barley Grain 19
Mean/Median Particle Size of Coarsely Dry-rolled Barley Grain 20
Conclusions and Implications 21
Acknowledgements 21
Tables.
Table 1 13
Table 2 15
Table 3 17
Table 4 18
Table 5 19
Table 6 20
PART I
Project 2: IN SITU DEGRADATION CHARACTERISTICS OF HYDROXY-CINNAMIC ACIDS AND FIBER OF VARIOUS BALREY VARIETIES 22

Project Details 22
Introduction 22
Objective 23
Materials and Methods 23
Barley Samples and Preparation 23
In Situ Rumen Incubation 23
Trial 1: Rumen Degradability of DM, FA, PCA, NDF, ADF and ADL of Six Barley Varieties at 12 and 24 h Rumen Incubation 23
Trial 2: In Situ Rumen Degradation Kinetics of Two Barley Varieties 24
Statistical Analysis 25
Results and Discussion 25
Trial 1: Rumen Degradability of DM, FA, PCA, NDF, ADF and ADL of Six Barley Varieties at 12 and 24 h of Rumen Incubations 25
Trial 2: In Situ Rumen Degradation Kinetics of Ferulic Acid and ρ-Coumaric Acid in Two Barley Varieties 28
Degradation Characteristics of DM 28
Rumen Degradation Characteristics of FA and PCA 29
Conclusions and Implications 32
Acknowledgements 32

Tables

Table 1 26
Table 2 28
Table 3 30
Table 4 31

Figures

Figure 1. 28
Figure 2. 30
Figure 3. 31

PART I

Project 3: INVESTIGATION OF RELATIONSHIPS BETWEEN ORIGINAL CONTENT OF BARLEY HULL, HYDROXYCINNAMIC ACIDS, FIBER, PARTICLE SIZES IN VARIOUS BARLEY GRAIN AND IN SITU RUMEN DEGRADABILITIES 33
Project Details 33
Introduction 33
Objective 33
Materials and Methods 34
Barley Samples 34
Chemical Analysis 34
Statistical Analysis 34
Results and Discussion 34
Correlation Analysis between Original Content of Hull, FA, PCA, Fiber, Particle Sizes and Rumen Indigestible Residues at 12 and 24 h

of Rumen Incubation........34
The Effects of Barley Hull........34
The Effects of FA and PCA, Ratio of PCA/FA and total of FA+PCA........38
The Effects of NDF, ADF, ADL, Hemicellulose and Cellulose........39
The Effects of Mean/Median Grain Particle Size........40
Multi-regression Analysis between Hull, FA, PCA Content, Mean/median Particle Size and Rumen Indigestible Residues at 12 and 24 h of Rumen Incubation........40
Conclusions and Implications........42
Acknowledgements........42
Tables
Table 1........36
Table 2........41

PART I: GENERAL CONCLUSIONS........43
Literature Cited........44

PART II
Project 4: PARTICLE SIZE, CHEMICAL PROFILE AND IN SITU RUMEN DEGRADATION KINETICS OF DRY MATTER, CRUDE PROTEIN AND STARCH AMONG SIX BARLEY VARIETIES: EVALUATION AND CORRELATION........53
Project Details........53
Introduction........53
Objective........53
Materials and Methods........53
Barley Varieties and Processing Method........53
Particle Size Analysis........54
Animal Work........54
Animals and Diets........54
Rumen Incubation........54
Chemical Analysis........54
Rumen Degradation Kinetics........54
Statistical Analysis........55
Results and Discussion........55
Comparison of Mean and Median Particle Size of Coarsely Dry-Rolled Barley Grains among Six Barley Varieties........55
Evaluation of Chemical Profile and In Situ Rumen Degradation Kinetics of Dry Matter, Crude Protein and Starch among Six Barley Varieties........57
Correlation between Mean and Median Particle Sizes and Degradation Rate and Extent of Dry Matter, Crude Protein and Starch among Barley Varieties........64

Conclusions 67
Acknowledgements 67
Tables
Table 1 56
Table 2 58
Table 3 60
Table 4 62
Table 5 65
Table 6 66
Figures
Figure 1. 64

PART II
Project 5: USING DIFFUSE REFLECTANCE FOURIER TRANSFORM INFARED SPECTROSCOPY (DRIFT) AND SYNCHROTRON-BASED FTIR MICROSPECTROSCOPY (SFTIRM) TO CHARACTERIZE SEED INHERENT STRUCTURE AMONG SIX BARLEY VARIETIES 67
Project Summary 67
Project Details 67
Introduction 67
Objective 68
Materials and Methods 68
Barley Varieties and Growth Condition 68
Sample Preparation for DRIFT Spectroscopy 68
DRIFT Spectroscopy 68
DRIFT Data Collection and Processing 68
Sample Preparation for SFTIR Microspectroscopy 69
Synchrotron-based SFTIR Microspectroscopy 69
SFTIRM Data Collection and Processing 69
Multivariate Spectral Analysis 69
Statistical Analysis 69
Results and Discussion 70
Using DRIFT Spectroscopy to Characterize and Compare Structure and Chemical Make-Up of Hull Samples from Six Barley Varieties 70
Using DRIFT Spectroscopy to Characterize and Compare Structure-Chemical Make-Up of Whole Seed Samples from the Six Barley Varieties 74
Using Synchrotron-Based FTIR Microspectroscopy (SFTIRM) to Characterize and Compare Structural And Chemical Make-Up of Seed Endosperm Tissue among Six Barley Varieties 77
Correlations between Mean and Median Particle Sizes, In Situ Degradation Features of DM, CP, and Starch and Mid-IR Spectral Characteristics among Barley Varieties 88

Using Mid-IR Spectroscopy with Multivariate Molecular Spectral Analysis to Discriminate and Classify Molecular Structure Difference of Barley Seed: Comparison among Barley Varieties 95
Conclusions 23
Acknowledgements 24

Tables

Table 1 73
Table 2 76
Table 3 83
Table 4 84
Table 5 85
Table 6 89
Table 7 90
Table 8 91
Table 9 92
Table 10 93
Table 11 94

Figures

Figure 1 71
Figure 2 75
Figure 3 78
Figure 4 80
Figure 5 96
Figure 6 100
Figure 7 104
PART II: GENERAL DISCUSSION AND CONCLUSIONS 108
General Discussion and Conclusions 108
Industry Application 109
Future Research 110
Literature Cited 110

Appendices 117
PART I 117
Appendix 1 118
Appendix 2 119
Appendix 3 119
Appendix 4 119
Appendix 5 122
Appendix 6 123
Table A1 118
Table A2 120
Table A3 122
Table A4 122
Table A5 123

Table A6. 123
Figure A1. 119

PART II 125
Appendix 1. 125
Figure A1. 126
Figure A2. 128
Figure A3. 130
Figure A4. 132
Table A1. 133

Highly Qualified Personal Training from This Project 134
Graduate Student Thesis One: Table of Contents 134
Graduate Student Thesis Two: Table of Contents 137
Technology Transfer, Research Publications and Presentations /Seminars 142

Abstract

This large research program was divided into two major parts. Each part contained several studies. The Part I: Hull, Ferulic Acid, Paracoumaric Acid Content and Particle Size Characteristics of Various Barley Varieties in Relation to Nutrient Availability in Ruminants". The Part II: "Ruminal Nutrient Availability and Inherent Structural Features of Six Barley Varieties Using in Situ Technique and Mid-IR Spectroscopy". The following are abstract from the Part I studies.

There are more than 50 varieties of barley, but 10 occupy 90% of the acreage. Western Canada accounts for 90% of the national barley acreage and Saskatchewan nearly 50%. The fibrous barley hull is the main reason for barley's low available energy relative to corn. Barley grain contains hydroxycinnamic acids (mainly ferulic acid (FA, 3-methoxy-4-hydroxy-cinnamic acid) and ρ-coumaric acid (PCA, (4-hydroxycinnamic acid))) which are cross-linked to polysaccharides, therefore, limit cell wall degradability in the rumen. Particle size of barley grain also affects the digestion of barley in the rumen. The objective of this study was to evaluate a set of barley varieties grown in Saskatchewan (Canada) and provided by Crop Development Center (CDC, Canada) and find a variety with low hull, FA, PCA and fiber content, while maintaining large particle size after mechanical processing, and having high nutrient availability.

Three studies were conducted to determine the content of barley hull, FA, PCA, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) in various barley varieties. Mean/median particle size of the barley grain after coarse dry-rolling was also determined. The relationships among these parameters and the digestibility of barley grain in ruminants were then assessed. Six barley varieties (AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey and CDC Cowboy) from samples grown in three years (2003, 2004 and 2005) were evaluated in each study.

The first study determined the original content of barley hull, hydroxycinnmaic acids (FA, PCA), NDF, ADF, ADL, and mean/median particle size of barley grain and evaluated the effects of barley variety. The results showed barley variety had a significant impact on the chemical and physical profiles of barley grain, with CDC Helgason and CDC Dolly showing relatively lower content of barley hull, FA, PCA, NDF, ADF, ADL, hemicellulose and cellulose, and moderate mean/median particle size, whereas McLeod and CDC Cowboy showed the opposite.

The second study involved two consecutive trials. Trial 1 was to assess differences in the in situ rumen degradability of dry matter (DM), hydroxycinnamic acids (FA, PCA), NDF, ADF and ADL at 12 and 24 h of rumen incubations. Results revealed that CDC Dolly consistently showed relatively lower rumen residues of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h, with McLeod being opposite. Barley variety displayed some effects on the digestibility of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h. Since CDC Dolly demonstrated relatively less content of hull, FA, PCA, NDF, ADF, ADL and mean/median particle size and higher rumen digestibility among the six barley varieties, while McLeod was the opposite, CDC Dolly and McLeod were selected for the third trial in order to compare differences in the rumen degradation kinetics of DM, FA and PCA. Trial 2 did not show significant differences in effective degradation of DM, FA, except for PCA. In general, CDC Dolly exhibited better degradability of DM, FA and PCA than McLeod.

The third study analyzed the correlation and regression between the original content of barley hull, hydroxycinnamic acids (FA, PCA), NDF, ADF, ADL and mean/median particle size in barley grain and rumen residual content of the corresponding parameters at 12 and 24 h of rumen incubation. Results showed that FA content in barley grain had a predominantly negative effect on DM degradability, while barley hull content affected the degradability of NDF and ADF.

In summary, the present studies show that hull and FA content in barley grain have negative effects on the degradability of barley grain in ruminants and also showed that CDC Dolly could be an ideal feed barley grain for ruminants due to its lower hull and FA content and higher rumen dry matter degradability.

The following are abstract from Part II studies.

Barley grain is one of the main sources of feed for ruminants in Canada. Although barley varieties may have similar chemical composition, they exhibit different rumen degradation characteristics and nutrient availabilities. These biological differences may be related to structural chemical make-up or structural features among the varieties. The objectives of this study were to use the *in situ* technique and two Mid-IR molecular spectroscopy techniques, Diffuse Reflectance Fourier Transform IR Spectroscopy (DRIFT) and Synchrotron-based Fourier Transform IR Microspectroscopy (SFTIRM) to determine ruminal nutrient availabilities and inherent structural features in the hull, seed and endosperm of the six barley varieties (AC Metcalfe, McLeod, CDC Dolly, CDC Helgason, CDC Trey, and CDC Cowboy) and to study the relationships between structural characteristics, mean and median particle size and nutrient availability. The nylon bag technique was used to incubate coarsely dry-rolled barley samples for 0, 2, 4, 8, 12, 24 and 48 h in the rumen of three mature Holstein dry dairy cows, which were ruminally cannulated. The rumen degradation kinetics of dry matter (DM), crude protein (CP) and starch were determined using first order degradation kinetics equations. Results indicated that there were significant differences in the mean and median particle size, degradation kinetics of each individual nutrient (DM, CP, and starch) among the six barley varieties. CDC Helgason showed the lowest degradation rate and extent of all nutrients (DM, CP, and starch) among the six barley varieties with larger particle size. Compared with other five varieties, CDC Helgason may be more suitable for ruminants feeding because of the lowest degradation rate and extent. The results also revealed a strong correlation between median particle size and the rate and extent of rumen degradation.

The results also showed that both DRIFT and SFTIR techniques associated with uni- and two multi-variate analyses were capable to efficiently discriminate and classify the inherent molecular structural features among the different varieties of barleys. Uni-variate analyses were conducted using both the DRIFT spectroscopy (hull and whole seed sample) and SFTIR microspectroscopy (endosperm tissue). The results from hull samples showed significant differences in the peak area of aromatic lignin, cellulosic compound, and total carbohydrates (CHO), and the ratio of lignin to cellulosic compound among the six barley varieties. The results from whole seed samples showed significant difference in the peak area and height of Amide I, peak area of total CHO and structural CHO (cellulosic), and the ratio of Amide I to total CHO area, and the ratio of total CHO to structural CHO. Significant differences were also found in the SFTIR results from endosperm tissue. With two multivariate spectral analysis techniques: Agglomerative hierarchical cluster (AHCA) and Principal component analyses (PCA) applied on whole seed sample, the CDC Helgason was distinguished from AC Metcalfe, CDC Dolly, McLeod and CDC Cowboy in fingerprint (1800-800 cm^{-1}) and CHO region (1185-800 cm^{-1}), from AC Metcalfe, McLeod and CDC Cowboy in protein region (1715-1485 cm^{-1}). Information from this study involving probing the seed internal structure of barley may provide a further insight as to why barley varieties exhibit different rumen degradations.

In conclusion, the CDC Dolly and CDC Helgason could be an ideal feed barley grains for ruminants due to its lower hull and FA content, higher rumen dry matter degradability and lower rate and extent of rumen degradation. Both the molecular spectroscopy techniques, DRIFT and Synchrotron based SFTIRM could be used to detect structural characteristics at cellular and molecular level in relation to nutrient utilization and availability. The information obtained from this proposed program aids barley breeding programs for selecting superior varieties of barley and for prediction of barley quality and nutritive value for humans and animals.

Executive Summary

This final report provides information on the completed status of this project. Chris (N.) Liu and Gali (L.) Du were hired as graduate students for this research program. The program was divided into two major parts. Each part contained several studies. The Part I: "Hull, Ferulic Acid, Paracoumaric Acid Content and Particle Size Characteristics of Various Barley Varieties in Relation to Nutrient Availability in Ruminants". The Part II: "Ruminal Nutrient Availability and Inherent Structural Features of Six Barley Varieties Using in Situ Technique and Mid-IR Molecular Spectroscopy".

In the Part I, we did three projects. We investigated chemical and physical differences among the various barley varieties grown in western Canada. We studied in situ degradation characteristics of hydroxycinnamic acids and fiber of various barley varieties. We also investigated the relationships between original content of hull, hydroxycinnamic acids, fiber, particle sizes in various barley grain and in situ rumen degradabilities.

Part I. Hull, Ferulic Acid, Paracoumaric Acid Content and Particle Size Characteristics of Various Barley Varieties in Relation to Nutrient Availability in Ruminants.

Project 1. Investigation of chemical and physical differences among the various barley varieties grown in western Canada (completed).

Project 2. In situ degradation characteristics of hydroxycinnamic acids and fiber of various barley varieties (completed)

Project 3. investigated the relationships between original content of hull, hydroxycinnamic acids, fiber, particle sizes in various barley grain and in situ rumen degradabilities. (completed)

In the Part II, we did two projects. In the first study, we studied the particle size, chemical profile and in situ rumen degradation kinetics of dry matter, crude protein and starch among six barley varieties: evaluation and correlation. In the second study, we used diffuse reflectance Fourier transform infared spectroscopy (DRIFT) and synchrotron-based FTIR microspectroscopy (SFTIRM) to characterize seed inherent structure among six barley varieties

Part II. Ruminal Nutrient Availability and Inherent Structural Features of Six Barley Varieties Using in Situ Technique and Mid-IR Molecular Spectroscopy .

Project 4. Particle size, chemical profile and in situ rumen degradation kinetics of dry matter, crude protein and starch among six barley varieties: evaluation and correlation. (completed).

Project 5. Using diffuse reflectance Fourier transform infared spectroscopy (DRIFT) and synchrotron-based FTIR microspectroscopy (SFTIRM) to characterize seed inherent structure among six barley varieties (completed)

This work and findings have been accepted or published as peer-reviewed full research articles by the Journal of Agricultural and Food Chemistry (two in press; one published), Cereal Research Communications (1 article In Press), Journal of Animal Physiology and Animal Nutrition. Journal of Cereal Science (1 article in press), Journal of Cereal Science (one in review). Three short articles were in Life Sciences Section, Canadian Light Source (CLS: Synchrotron) Activity Report 2009, 2008 and 2007 (see following publication list section for details).

We have been invited to present our research work in the upcoming The 6th Canadian Barley Symposium (CBS) in July this year. The research results presented in various professional and industry meetings: Canadian Society of Animal Science (CSAS) Annual Conference, Canadian

Nutrition Congress (CNC), Canadian Seed Growers' Association Annual Conference, Soils and Crops Conference, Inaugural American Society of Animal Science (ASAS)-CAAVM Pacific Rim Conference, the VII International Symposium on the Nutrition of Herbivores (ISNH7) and the Canadian Light Source Annual Users' Meetings (see following presentation list section for details)

Research Progress

Introduction: Research Motivation and Background

There are more than 50 varieties of barley, but 10 occupy 90% of the acreage. Western Canada accounts for 90% of the national barley acreage and Saskatchewan nearly 50%. The vast majority of barley varieties are hulled. In Canada, approximately 12-14 million metric tonnes of barleys are produced annually (Agriculture and Agri-Food Canada, 2000), most of which is produced in western Canada. The hull represents approximately 10-12% of the weight of the barley seed. This leaves in excess of more than one million metric tonnes of barley that is of low feed quality. Barley hulls, a relatively indigestible portion of the barley grain, are the main reason for its lower energy relative to corn. Nutritionally, barley hulls are high in fiber and low in protein. Due to the large supply of barley as a feed, it is economically important to improve the nutritional qualities of barley hulls.

Barley hulls contain hydroxycinnamic acids, mainly ferulic acid (3-methoxy-4-hydroxy-cinnamic acid) and para-coumaric acid (4-hydroxycinnamic acid), which are covalently cross-linked to polysaccharides by ester bonds and to components of lignin mainly by ether bonds. These hydroxycinnamic acids are produced via the phenylpropanoid biosynthetic pathway. These cross-links are a barrier to biodegradation and limit cell-wall degradability by rumen microorganisms. It is believed that these hydroxycinamic acids are the factor most inhibitory to the biodegradability of plant cell wall polysaccharides.

Overall Project Objectives:

1. The objectives of this feed research program are to determine genotypic differences in total content and concentration of hydroxycinnamic acids (Ferulic and para-coumaric acids), total hull content, rumen degradation kinetics of hydroxycinnamic acids, and structural-chemical features among various varieties of barley.
2. The goal is to investigate and select a superior variety of barley (we call it "DOUBLE LOW BARLEY": low hull content and low content of hydroxycinnamic acids in the hulls) with a high nutritive value for the Saskatchewan feed and livestock Industry.
3. The other objective is to increase basic knowledge of the nutritional relevance of barley intrinsic structure and to apply this information to the production of high quality feeding programs.
4. The information obtained from this proposed program will aid barley breeding programs for selecting superior varieties of barley and for prediction of barley quality and nutritive value for humans and animals.
5. Highly qualified personnel training: Train two graduate students.

Completed Projects

PART I

Project 1: INVESTIGATION OF CHEMICAL AND PHYSICAL DIFFERENCES AMONG THE VARIOUS BARLEY VARIETIES GROWN IN WESTERN CANADA

Project Details

Introduction

Barley is one of the principal feed grains for ruminants in western Canada. Approximately 10-14 million tonnes are produced annually in Canada (more than 90% of which are from western Canada), and over half of the national barley yield is used by the feedlot sector (Statistics Canada, 2000-2008). There are more than 50 barley varieties grown in western Canada, most of which are hulled two-row varieties (Brian G. Rossnagel, University of Saskatchewan). On average the barley hull represents roughly 13% of the grain by weight (Evers et al. 1999) which means that over one million tonnes of barley hull is produced annually. Moreover, barley hull is extremely fibrous (28% cellulose, 34% hemicellulose and 23% lignin) and low in valuable nutrients (Olkku et al. 2005). Nutritionally, the barley hull is indigestible for monogastric animals and only partially digestible for ruminants. The low digestibility of barley hull limits nutrient and energy availability to animals. Although barley contains higher protein than corn, it fails to compete in term of digestible energy, primarily due to the presence of the hull. However, the hull is an important component that may benefit ruminants by slowing down barley starch fermentation in the rumen and reducing metabolic diseases.

Due to the major usage of hulled barley by the malting and the cattle industries, it is economically important to develop a barley variety with less hull content and improved nutritional quality. In addition to lignin, researchers have found that hydroxycinnamic acid-carbohydrate complexes are another important inhibitory factor related to plant cell wall digestibility in ruminants. In barley and other cereal grains, FA and PCA are two major low molecular weight hydroxycinnamic acids (Nordkvist et al. 1984; Hernanz et al. 2001). FA and PCA are concentrated in the cell walls of the outer coverings of barley seeds, mainly in the bran (Nordkvist et al. 1984; Sancho et al. 2001). In gramineous plants such as barley, FA is covalently linked to cell wall polysaccharides (especially arabinoxylans) by ester bonds and to lignin mainly by ether bonds (Gubler et al. 1985; Sun et al. 2002). Through ester and ether linkages, FA is extensively involved in cross-linking between plant cell wall polysaccharides and lignin (Iiyama et al. 1990; Iiyama and Lam 2001; Sun et al. 2002). Furthermore, FA can dimerize and trimerize through oxidative coupling (Bunzel et al. 2001; Hernanz et al. 2001; Bunzel et al. 2005). Therefore, FA forms intra- and/or inter- molecular ester–ether bridges between lignin and cell wall polysaccharides. Although the role of FA in the digestibility of plant cell walls is not well elucidated, the proposed mechanism behind its negative effect on digestibility of cell wall polysaccharides is that FA cross-linkages increase steric interference to rumen microbial digestion and shield bound polysaccharides from enzymatic hydrolysis (Jung and Deetz 1993; Grabber et al. 1998).

PCA is mainly esterified and etherified to lignin in plant cell walls, and seldom linked to polysaccharides (Ralph and Helm 1993; Sun et al. 2002). Since PCA does not form ester-ether cross-linkages as FA, it is not considered to be directly involved in plant cell wall digestibility and primarily functions as an indicator of cell wall lignification (Jung and Allen 1995; Grabber et al. 2004). FA and PCA content in barley may be influenced by barley variety, growing environment, disease and agronomic management (Miyamoto et al. 1994; Zupfer et al. 1998; Blokker et al. 2006).

It is a common practice in the western Canada feedlot to coarsely process barley grain to breach the tough barley hull before feeding, thereby improving digestibility in the rumen. However, over-processing can lead to an unpalatable ration and reduce DM intake. It can also cause digestive problems such as acidosis, laminitis and liver abscesses due to the rapid rumen fermentation of starch. Larger particle size can reduce the surface area for microbial colonization and enzymatic attack, which in turn slows down the rate of barley starch degradation in the rumen without affecting the extent of digestion. Therefore, larger grain particle size is preferred in order to maximize barley grain digestibility, animal intake and performance (Mathison 1996; Beauchemin et al. 2001). Barley particle size reduction obtained after dry-rolling is related to grain hardness, texture, and composition (Camm 2008; Darlington et al. 2001; Zhang et al. 2003; Psota et al. 2007). Mean/median particle size can be used to describe the differences in particle size among various barley varieties after mechanical processing. In order to properly predict mean/median particle size for coarse dry-rolled grain, a suitable model is required. Pond et al. (1984) and Fisher et al (1988) proposed different mathematic equations for analyzing mean/median particle size of substances with irregular shapes.

Objectives

The main objectives of this study were 1) to determine the content of hull, FA, PCA, NDF, ADF and ADL in six barley varieties from three consecutive years; 2) to determine the mean/median particle size of barley grain obtained after coarse dry-rolling.

Materials and Methods

Barley Varieties and Samples

Barley varieties used in this project were obtained from the Crop Development Centre (CDC) and included AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey and CDC Cowboy (Table 1). These were two-row malting or feed barley varieties, harvested from three different years (2003, 2004, 2005), and grown at the Kernen Crop Research Farm, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Table 1. Barley samples used in the project

Barley variety	Barley type	Year		
AC Metcalfe	Two-row, Malting	2003	2004	2005
McLeod	Two-row, Feed	2003	2004	2005
CDC Dolly	Two-row, Feed	2003	2004	2005
CDC Helgason	Two-row, Feed	2003	2004	2005

CDC Trey	Two-row, Feed	2003	2004	2005
CDC Cowboy	Two-row, Feed (Forage)	2003	2004	2005

Determination of Barley Hull Content Using Modified European Brewery Convention (EBC) Method

The modified EBC method (Appendix 1) was used to analyze the hull content in the six barley varieties from three years. Barley grain (20 g) was boiled and digested for 3 min in a solution of 80 ml sodium hypochlorite (12%) (ClearTech Industries Inc., Saskatchewan, Canada) and 20 ml sodium hydroxide (3.125 N) (pellet, VWR International, Pennsylvania, USA). The dehulled samples were then dried and ground to determine barley hull content.

Determination of the Content of NDF, ADF and ADL

Eighteen barley samples (6 varieties × 3 years) were analyzed for NDF, ADF and ADL. Whole barley grain was ground through 1 mm pore-size mesh screens (Retsch ZM-100, Brinkmann Instruments Ltd., Ontario, Canada). Ground barley (0.5 g) was weighed and placed in F57 filter bags (Ankom Technology Corporation, Fairport, NY). All samples were treated with alpha-amylase (Anachemia Science, Anachemia Canada Inc. Winnipeg, MB) in 8 M urea (pellet, VWR International, Pennsylvania, USA) solution overnight (Van Soest et al. 1991). Alpha-amylase was used at 100 µl per 30 ml 8 M urea. After incubation, all bags for NDF analysis were rinsed ten times with warm tap water. NDF and ADF were determined in tandem using Ankom Fibre Analyzer (Ankom Technology) by boiling samples in neutral detergent solution (Ankom Technology) for 75 min and acid detergent solution (Ankom Technology) for 60 min, respectively (Van Soest et al. 1991). ADL was determined by oxidizing and removing the remaining carbohydrate residue in ADF with 72% H_2SO_4 (98%, VWR International, Pennsylvania, USA) (AOAC 1990).

Determination of Ferulic Acid and ρ-Coumaric Acid Content

Sample Preparation:

Whole barley grain was ground through a 0.5 mm pore-size mesh screen followed by a 0.25 mm screen using a Retsch ZM–1 grinder (Brinkmann Instruments Canada Ltd, Ontario).

Alkaline hydrolysis of barley samples and extraction of FA and PCA were based on the method of Hernanz et al. (2001) with some modifications (Appendix 2). Ground barley grain (50 mg) was mixed with 0.75 ml 1% (w/v) α-amylase in a 0.05 M phosphate solution (pH 6.9) and incubated in a hot water bath (90 °C) for 1 h. The samples were then cooled at room temperature and centrifuged at 14,000 rpm for 20 min. The supernatant (S1) was collected and stored at -20 °C. The precipitated pellets were hydrolyzed by adding 2 M NaOH solution (0.55 mL) followed by incubation at ambient temperature for 16 h in the dark (samples were wrapped with aluminium tinfoil). After centrifugation (14,000 rpm, 10 min), the supernatant (S2) was collected and combined together with the initial supernatant (S1), acidified with 200 µl 6 M HCl to pH 2, and then extracted five times with equal volumes of ethyl acetate. The organic solutions were combined and evaporated until dry under N_2 in a heat block at 40 °C. The residue was dissolved in 1 mL of methanol/water (50:50, v/v) and filtered through a 0.45 µm syringe filter (Minipore, Bedford, USA) and 5 µl samples were analyzed by HPLC. All samples were prepared and analyzed in triplicate.

HPLC Condition:

HPLC analysis of ferulic acid (FA, trans-4-Hydroxy-3-methoxycinnamic acid) (46278) and ρ-coumaric acid (PCA, trans-4-Hydroxycinnamic acid) (C9008) were developed based on the method of Olkwoski et al. (2003) with some modifications. An Agilent 1100 series HPLC system was used, which consists of a system controller (HP Chem Station computer program), pump, auto-sample processor and photo diode array detector (DAD) (Interface 35900E). Separation was performed by isocratic elution with a mobile phase of 5.5% methanol, pH 8.0, and 20 mM K_2HPO_4-KH_2PO_4 in a reverse phase PRP-1 column (Hamlton, 150×4.6 mm, 5 μm, pH 1-13) at room temperature. The isocratic elution flow rate was 1 ml/min and samples (5 μl) were introduced into the column using an auto-sampler. The detection was monitored at 305 nm. FA and PCA in samples were identified by comparison of retention time and DAD-UV spectra with that of standard compounds and were quantified using the external standards. HPLC chromatogram is shown in Appendix 3. FA and PCA concentrations of sample extracts were extrapolated from the FA and PCA standard curves. The standards were prepared as stock solution at 2 mg/ml in methanol. Calibration curves were calculated on the basis of the linear correlation between concentration of standards and the area of the FA and PCA peaks. All samples were analyzed in triplicate.

Determination of Mean/Median Particle Size of Barley Grain Obtained after Dry-rolling

Barley samples were coarsely dry-rolled in a grain roller mill (Seven Grain Mill, Apollo Machine and Products Ltd., Saskatoon, Canada) in the College of Engineering (University of Saskatchewan, Canada) through a 1.55 mm gap. Particle size distribution of these cracked samples was determined by weight distribution. In brief, triplicate samples (100 g) were sifted through a stack of six test sieves plus one bottom pan arranged in descending sizes of square sieve apertures (Table 2), fitted in a Ro-Tap sieve shaker (Tyler Industrial Products, USA). The duration of sieving (rotation and tapping) was determined by sieving initially for 1 min and increasing to 5 min until sifting had reached equilibrium according to the American National Standards Institute (ANSI 2003) sieving method.

Table 2. Aperture sizes for test sieves

Sieve No.	Aperture Size (mm)
6	3.36
8	2.36
12	1.70
16	1.19
20	0.84
30	0.58

After sieving, the fractions remaining on each screen were weighed, and particle size distribution was expressed in percentage cumulative weight oversize by adding up the weight on each sieve and those from all larger screens (ANSI 2003). The mean/median particle size values were estimated by fitting these data into Pond's equation (3.1) with 0 mm = 100% (Pond et al. 1984) (0 mm = 100% means that particles passing the 0.58 mm sieve are included in Pond's equation). The selection of this equation is explained in the Appendix 4. Data were computed using the NLIN procedure of the Statistical Analytical System (SAS) (SAS Institute, Inc. 2002).

Pond's Equation:

$R = 100\ e^{-k(s-w)}$

Mean particle size = $1/k+w$

Median particle size = $0.693/k+w$.

Where:

s = sieve opening size (mm);

w = the smallest predictable particle size;

k = the decay constant of the exponential curve describes the proportionality constant between the percent of particles passed to the next sieve and the percent remained.

Statistical Analysis

Analysis of variance (ANOVA) was performed using the Proc Mixed procedure of SAS (2002). Experiments were carried out as a completely randomized design (CRD) with barley variety as a fixed effect and year as replication. Treatments were compared by the LSD test. Significance was declared at $P < 0.05$.

The model used for the analysis was:

$$Y_{ij} = \mu + t_i + e_{ij}$$

Where Y_{ij} is an observation of the dependent variable; μ is the overall mean; t_i is the fixed effect of the *i*th barley variety ($i = 1 - 6$); and e_{ij} is the error term.

Results and Discussion

Variety Effect on Barley Hull Content

The hull content for the six barley varieties (AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey and CDC Cowboy) collected over three years (2003, 2004, 2005) varied from 9.4 to 10.7% with a mean value of 10.1% (Table 3). Significant effects of variety on hull content were detected ($P<0.05$), with McLeod and CDC Cowboy demonstrating the highest hull content, and CDC Dolly and CDC Helgason showing the lowest. The hull content in this experiment agreed with that reported by Evers et al. (1999) who indicated that barley hull content varies from 7 to 25% among two-row and six-row barley grain, while two-row barley commonly displays lower hull content with a mean content of 10.4%. Barley hull content is influenced by both environment as well as genetic factors. Evers et al. (1999) stated that barley growing in higher latitudes produces less hull. Canada is located in the very northern latitudes where relatively low temperature prevails during the growing season and tends to grow barley with low hull content. Fox et al. (2006) observed that hull content was associated with a genomic region on barley chromosome 2H. Such information provides the genetic basis for the variety effect on hull content.

Although CDC Dolly is not a new variety, it is still widely cultivated for feed barley in Canada, and is often used as a reference variety for barley breeders. CDC Dolly usually produces a heavier test weight than many other varieties (Government of Alberta 2007). The results in this study indicate that CDC Dolly had a lower ($P<0.05$) hull content than McLeod and CDC Cowboy, but similar to CDC Helgason, CDC Trey and AC Metcalfe, while CDC Helgason had the lowest ($P<0.05$) hull content compared to the other barley varieties.

Table 3. Variation of variety effect on hull content in six barley varieties

Barley variety	Hull content (%DM)
McLeod	10.7^{a}
CDC Cowboy	10.4^{ab}
AC Metcalfe	10.2^{bc}
CDC Trey	10.1^{bc}
CDC Dolly	9.8^{cd}
CDC Helgason	9.4^{d}
SEM	0.11

$^{a, b, c, d}$ Different superscripts of in the same column are significantly different ($P < 0.05$).

CDC Cowboy is a barley variety oriented toward forage value and is quite low in grain yield production (Government of Alberta 2007). However, CDC Cowboy actually performed fairly well in kernel weight test, far better than CDC Helgason which was low in kernel weight test and high in yield test (Government of Alberta 2007). Therefore, it is reasonable to expect higher hull content in CDC Helgason than in CDC Cowboy. However, the present study has revealed the reverse results. Such inconsistencies between barley hull content and kernel weight, grain size or plumpness (Field Crop Development Centre and Lacombe Research Centre 2006; SeCan 2006; Government of Alberta 2007) implies that some other factors are involved in affecting barley hull content, such as genetic features, climate, agronomic management, disease resistance and soil adaptation (Evers et al. 1999; Fox et al. 2006). More research is needed to investigate factors determining barley hull content. In the present study, the confounding factors such as weather, environment and agronomic management have been managed by applying the same agronomic practices in the same year for all the six varieties, so variety should have exerted its maximum effect on the difference of hull content in the barley samples.

Variety Effect on the Content of FA, PCA and PCA/FA in Barley Grain

Barley grain contains two major low molecular weight hydroxycinnamic acids: FA and PCA (Nordkvist et al. 1984; Chemey et al. 1992). They are covalently cross-linked to barley cell wall polysaccharides and lignin by ester and/or ether bonds (Iiyama et al. 1990; Lam et al. 1992a; Hernanz et al. 2001) and directly or indirectly involved in affecting the digestibility of cell wall polysaccharides (Jung and Allen 1995; Grabber et al. 2004). About 80% of FA and PCA is found in the bran cell walls and the rest in the cell walls of the endosperm (Hernanz et al. 2001). Rumen microorganisms are able to synthesize limited phenolic acid esterases to ultimately break down the ester bonds. The ether linkages however are difficult to cleave in the rumen anaerobic environment (McSweeney et al. 1994; Jung and Allen 1995). Therefore, only the esterified FA and PCA were analyzed in the present study. The esterified FA and PCA are alkaline sensitive and can be released by mild alkaline hydrolysis (2 N NaOH) at room temperature and are usually analyzed by HPLC (Zupfer et al. 1998; Hernanz et al. 2001; Yu et al. 2002; Holtekjolen et al. 2006).

Table 4 shows the effects of barley variety on the content of FA and PCA, and ratio of PCA to FA

(PCA/FA) in this study. Significant variety effects were detected ($P<0.05$). In all samples, barley grain contained a higher FA content than PCA, ranging from 555 to 663 μg/g DM for FA, and 283 to 345 μg/g DM for PCA. Accordingly, the ratio of PCA to FA varied from 0.49 to 0.56 (or FA/PCA 1.8 to 2.1). Hernanz et al. (2001) examined several European malting and feed barley varieties and found a range of 359 to 624 μg/g DM for FA content, 79 to 260 μg/g DM for PCA content, and 0.27 to 0.37 for ratio of PCA/FA. Holtekjolen et al. (2006) studied five varieties of hulled two-row barley grown in Norway in 2002, and observed that FA content varied from 512 to 723 μg/g DM, PCA content varied from 114 to 244 μg/g DM, and the ratio of PCA/FA varied from 0.16 to 0.48. FA content in the present study was similar to their findings, but PCA content was slightly higher in our barley samples; consequently, the ratio of PCA/FA was also higher. This is likely a result of the different growing environments and varieties studied.

Table 4. Variation of variety effect on the content of FA, PCA and ratio of PCA/FA of whole barley grain in six barley varieties

Barley variety	FA (μg/g DM)	PCA (μg/g DM)	PCA/FA
McLeod	663 ^{a}	345 ^{a}	0.52 ^{ab}
CDC Cowboy	606 ^{b}	339 ^{a}	0.56 ^{a}
AC Metcalfe	594 ^{bc}	308 ^{b}	0.52 ^{ab}
CDC Helgason	581 ^{bcd}	283 ^{b}	0.49 ^{b}
CDC Trey	563 ^{cd}	301 ^{b}	0.53 ^{a}
CDC Dolly	555 ^{d}	306 ^{b}	0.55 ^{a}
SEM	13.4	10.1	0.015

$^{a, b, c, d}$ Different superscripts of in the same column are significantly different ($P < 0.05$).

In plant cell walls, FA is esterified to both polysaccharides and lignin, and mainly etherified to lignin (Jung and Deetz 1993; Morrison et al. 1998; Iiyama and Lam 2001; Sun et al. 2002). Through radical coupling reactions, FA forms cross-linkages between cell wall polysaccharides and lignin and between polysaccharides (Iiyama et al. 1994; Ralph et al. 1995). The ferulate cross-linkages strengthen plant cell walls to defend against pathogenic microorganisms and microbiological degradation (Kroon and Williamson 1999). It is of special interest in ruminants because ferulate linkages limit the digestibility of the plant cell wall in the rumen by forming a steric obstacle to degradation by rumen bacteria (Moore and Jung 2001). FA in barley grain is mostly concentrated in barley bran (Nordkvist et al. 1984; Hernanz et al. 2001). Comparison of FA content among the six varieties shows that barley variety significantly influenced the FA content in barley grain ($P<0.05$). McLeod exhibited the highest ($P<0.05$) FA content, while CDC Dolly, CDC Trey and CDC Helgason were significantly lower ($P<0.05$) in FA content. Zupfer et al. (1998) also observed significant variety differences of FA content in barley grain. At the same time, they reported a strong genetic basis for the difference of FA content in barley, but did not observe a relationship between kernel weight and FA content.

PCA content among the six barley varieties was also significantly different ($P<0.05$). The ranking sequence showed that McLeod and CDC Cowboy had the highest PCA content ($P<0.05$), but statistical difference of PCA content among AC Metcalfe, CDC Dolly, CDC Trey, and CDC Helgason was not observed. PCA is mainly esterified and etherified to cell wall lignin and seldom linked to polysaccharides, so it is known as a good indicator of plant cell wall lignification (Jung and Allen 1995; Sun et al. 2002; Grabber et al. 2004). More PCA indicates more lignified plant cell walls (Jung and Allen 1995; Grabber et al. 2004). Barley bran, especially the hull is the most lignified tissue in barley grain. It is possible that PCA content may relate to barley hull content or the degree of lignification in the hull. Current results show McLeod and CDC Cowboy had the highest ($P<0.05$) content of PCA which was in accordance with that of barley hull content.

The ratio of PCA/FA is proposed as an indicator for plant tissue lignification, with limited lignified plant tissues having a low ratio and a high ratio indicating an even distribution of lignification in plant tissues (Grabber et al. 2004). In the present study, CDC Helgason had a lower ($P<0.05$) PCA/FA than CDC Cowboy, CDC Dolly and CDC Trey. Correspondingly, in the comparison of barley hull content, CDC Helgason also showed signifcantly lower hull content than CDC Cowboy and CDC Trey, but was similar to CDC Dolly. Further study is required to assess the relationship among the FA and PCA content and hull content in barley grain.

NDF, ADF, ADL, Hemicellulose and Cellulose in Barley Grain

NDF, ADF, ADL, hemicellulose and cellulose of the six barley varieties are presented in Table 5. For all the five parameters, variety had a significant influence ($P<0.05$). NDF values varied from 17.6 to 21.9 %DM with a mean of 19.5 %DM. ADF in barley grain was much lower than NDF, with a range from 5.5 to 7.0 %DM and mean of 6.0 %DM. ADL varied from 1.7 to 2.1 %DM with an average of 1.9 %DM. By difference, the contents of hemicellulose (NDF-ADF) and cellulose (ADF-ADL) were 13.5 %DM (from 12.2% to 14.9 %DM) and 4.1 %DM (from 3.8 to 4.9 %DM), respectively.

Table 5. Variation of variety effect on the content of ADF, NDF, ADL, hemicellulose and cellulose in six barley varieties

Variety	Original Samples				
	NDF (%DM)	ADF (%DM)	ADL (%DM)	Hemicellulose (%DM)	Cellulose (%DM)
McLeod	21.9^{a}	7.0^{a}	2.1^{a}	14.9^{a}	4.9^{a}
CDC Cowboy	20.5^{b}	6.4^{b}	2.1^{ab}	14.0^{ab}	4.4^{b}
AC Metcalfe	19.7^{bc}	5.7^{cd}	2.0^{bc}	13.9^{bc}	3.8^{c}
CDC Trey	19.0^{cd}	5.9^{c}	1.8^{cd}	13.1^{cd}	4.0^{c}
CDC Dolly	18.2^{de}	5.5^{cd}	1.8^{d}	12.6^{de}	3.7^{c}
CDC Helgason	17.6^{e}	5.5^{d}	1.7^{d}	12.2^{e}	3.8^{c}
SEM	0.41	0.13	0.05	0.30	0.12
Mean	19.5	6.0	1.9	13.5	4.1

$^{a, b, c, d}$ Different superscripts of in the same column are significantly different ($P < 0.05$); NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, Hemicellulose = NDF - ADF, and Cellulose = ADF - ADL.

In general, McLeod and CDC Cowboy had higher ($P<0.05$) fiber content than the other barley varieties. CDC Helgason and CDC Dolly were relatively low in fiber content among the six examined barley varieties and did not differ from each other. In the present study, NDF and ADF content in the barley grain were slightly lower than the results from Fairbairn et al. (1999), while ADL was in accordance with NRC (2001). Studies have reported a wide variation in fiber content in barley, with NDF from 12 to 26 %DM, ADF from 4 to 8 %DM (Henry 1988 cited by Fairbairn et al. 1999) and ADL at approximately 1% (Fairbairn et al. 1999). NRC (2001) reported that NDF in barley grain was a 20.8 ± 8.6 %DM; ADF was 7.2 ± 2.8 %DM; and ADL was 1.9 ± 1.1 %DM. The calculated mean of hemicellulose was 13.6 %DM and the mean of cellulose was 5.3 %DM from NRC (2001), which are comparable to our results. All of this information implies that the fiber content varies in different barley varieties.

Ruminants are able to digest and utilize hemicellulose and cellulose as energy sources. Nonetheless, hemicellulose and cellulose contain less digestible energy than lipid, starch and protein. Therefore, barley grain containing more hemicellulose and cellulose will have lower energy density and digestibility. McLeod and CDC Cowboy contained more fiber content than the others and may not be good feed barley for ruminants. In contrast, CDC Helgason and CDC Dolly with less fiber content are better choices for feed barley as they would provide higher digestible energy at the same feeding level. Lignin, an important structural composition of plant cell walls, is attached to hemicellulose and cellulose and concentrated in the secondary plant cell walls (Rowell et al. 2000). Lignin is not carbohydrate and cannot be utilized as the energy source. Furthermore, no apparent lignin-degrading microorganisms or enzymes in the rumen can degrade lignin efficiently (Van Soest 1994). As a result, lignin is well-known as an inhibitor of plant cell wall digestibility. CDC Helgason and CDC Dolly with lower content of lignin may be a good choice for animal feed.

Mean/Median Particle Size of Coarsely Dry-rolled Barley Grain

As illustrated in Table 6, barley variety exerted a significant effect on the mean/median particle size estimated from Pond's equation with 0 mm = 100% ($P<0.05$). The range of mean particle size estimated using Pond's equation was from 3.06 to 3.66 mm with an average value of 3.35 mm. Median particle size behaved similarly ranging from 2.71 to 3.04 mm and an average of 2.91 mm. Numerically, the predicted sequence for mean particle size from large to small was CDC Cowboy, CDC Helgason, McLeod, CDC Dolly, AC Metcalfe, CDC Trey, while in the rank of median particle size, it was CDC Cowboy, CDC Helgason, CDC Dolly, McLeod, AC Metcalfe, CDC Trey.

Table 6. Variation of variety effect on mean/median particle sizes of coarsely dry-rolled barley predicted by Pond's equation with 0 mm = 100% in six barley varieties

Barley Variety	Mean (mm)	Median (mm)
CDC Cowboy	3.66^{a}	3.04^{a}
CDC Helgason	3.39^{ab}	2.98^{a}
McLeod	3.35^{bc}	2.92^{a}
CDC Dolly	3.33^{bc}	2.94^{a}
AC Metcalfe	3.31^{bc}	2.84^{ab}
CDC Trey	3.06^{c}	2.71^{b}
SEM	0.073	0.047
Mean	3.35	2.91

[a, b, c, d] Different superscripts of in the same column are significantly different ($P < 0.05$).

Particle size reduction after mechanical processing is related to the grain's physical and chemical characteristics such as hardness. Camm (2008) reported that milling energy consumption had a positive relationship with barley endosperm hardness. Their results of the milling energy requirement and Single Kernel Characterization System (SKCS) hardness test from high to low were McLeod, CDC Dolly, CDC Helgason, and CDC Trey for the milling energy requirement, and McLeod, CDC Dolly, CDC Trey, and CDC Helgason for SKCS hardness test. In the present study, mean/median particle size of the same four barley samples demonstrated the similar trend of grain hardness with that of Camm (2008), with the exclusion of CDC Helgason. The similar trend potentially means that the particle size reduction of dry-rolled barley grain is related to grain hardness or milling energy consumption. However, in the present study, the significant difference in Pond's mean/median particle size was detected only between CDC Cowboy, CDC Helgason, and CDC Trey, while no difference ($P>0.05$) was observed among CDC Helgason, McLeod, CDC Dolly and AC Metcalfe. The variety difference of barley grain is responsible for the intrinsic chemical composition (e.g. β-glucan, protein) and grain hardness (Andersson et al. 1999; Izydorczyk et al. 2003; Caldwell et al. 2004), which consequently influences barley particle size distribution after mechanical manipulation. Fairbairn (1999) observed a significant difference in particle size among 20 barley varieties even when grain was finely ground. Bowman et al. (2001) observed that NDF content could affect the particle size reduction of barley grain.

Conclusions and Implications

The results of chemical and physical analyses show that barley variety had a significant effect on the content of hull, FA, PCA, NDF, ADF and ADL in various barley varieties, and also on the mean/median grain particle size obtained after coarse dry-rolling. Therefore, barley variety plays an important role in determining the quality of barley as a feed.

Generally, the varieties of McLeod and CDC Cowboy consistently contained higher content of hull, FA, PCA, NDF, ADF and ADL compared to CDC Dolly and CDC Helgason. Therefore, from a nutritional point of view, CDC Dolly and CDC Helgason are more valuable than McLeod and CDC Cowboy. But when mean/median particle size obtained after coarse dry-rolling was compared, CDC Cowboy and CDC Helgason showed larger particle size, and therefore become more promising as feed barley. On the whole, CDC Dolly and CDC Helgason have lower content of hull, FA, PCA, NDF, ADF and ADL, and have moderate mean/median particle size after dry-rolling, so both are good candidates for feed barley according to the current study. However, the best way to predict barley quality is in situ rumen degradation experiment. Therefore, further experiments are needed to investigate the exact relationship between in situ digestibility and the physical, chemical measurements in these barley varieties.

Acknowledgements

The authors would like to thank Saskatchewan Agriculture Development Fund (ADF, Project # 20040495) for financing this project and Saskatchewan Agriculture Development Fund (ADF) for Ministry of Agriculture Strategic Feed Research Chair Program.

PART I

Project 2: IN SITU DEGRADATION CHARACTERISTICS OF HYDROXY-CINNAMIC ACIDS AND FIBER OF VARIOUS BALREY VARIETIES

Project Details

Introduction

Barley grain is the principal energy source for cattle in western Canada. Barley seed contains mainly starch and protein which can be quickly fermented in the rumen. However, barley grain cell walls, especially in the bran consist of mainly fiber and other minor but important chemical components such as ferulic acid (FA) and ρ-coumaric acid (PCA), the presence of which could exert considerable effects on the rate and extent of grain degradation. It is well established that fiber is the major contributor to the relatively lower energy density in barley grain than in corn. Lignin is recognized as the most inhibiting constituent of plant cell wall with respect to digestion in animals. Among the physical fractions of barley grain, the hull is the most fibrous part, which contains roughly 85% fiber (Olkku et al. 2005). Therefore, barley hull is the most indigestible fraction.

Hydroxycinnamic acids (mainly FA and PCA) have been reported to adversely affect plant cell wall digestibility in ruminants. FA and PCA are the two principal hydroxycinnamic acids in barley grain, concentrated in the cell walls of the bran (Nordkvist et al. 1984; Chemey et al. 1992). FA and PCA are covalently linked to plant cell wall polysaccharides by ester bonds and to lignin by both ester and ether bonds (Iiyama et al. 1990; Lam et al. 1992a; Hernanz et al. 2001). However, FA is considered to have direct inhibitory influence on the digestibility of plant cell walls, while PCA is regarded primarily as an indicator of the degree of plant cell wall lignification (Jung and Allen 1995; Anderson and Schroeder 1999; Grabber et al. 2004).

Particle size is another important factor influencing barley digestion in the rumen. Whole barley grain fed to animals was shown to have significantly lower grain digestibility and animal performance compared to processed barley grain (Mathison 1996). In contrast to small particle size, large particle size reduces the surface area for rumen bacteria and enzymes to attack and digest, and accordingly slows down the fermentation rate of starch and digestion rate of barley grain in the rumen. It is also likely that particle size affects the extent of feed digestion by changing the digestion site and passage rate of feed in the digestive tract of cattle (Koenig et al. 2003; Rémond et al. 2004)

Feed degradability can be measured by using the in situ rumen nylon bag technique which reflects a more realistic rumen environment than in vitro and other methods. The first-order digestion kinetics equation (Ørskov and McDonald 1979) is the most widely applied model to describe the digestibility of individual feed components such as DM, protein, starch and fiber. With respect to in situ rumen degradability of DM, different varieties of barley grain demonstrated varying rate and extent of rumen degradation due to differences in the intrinsic physical structures and chemical components (Yu et al. 2003; Walker 2007). In practice, the in situ rumen digestibility of feed components varies due to many influencing factors such as feed processing, experimental techniques and animal status (Mustafa 1996; Vanzant et al. 1998). However, the in situ rumen nylon bag technique is still a widely used and useful method to compare and estimate feed quality.

Objectives

The specific objectives of this study were 1) to investigate in situ rumen digestibility of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h rumen incubation among the six varieties; 2) to select the relatively better and worse barley varieties based on the initial comparison study of hull, FA, PCA, NDF, ADF, ADL, particle size and rumen digestibility to further analyze and compare the rumen digestion kinetics of DM, FA and PCA.

Materials and Methods

Barley Samples and Preparation

Barley samples used in this experiment were the same six barley varieties used in the Chapter 3: AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey and CDC Cowboy from 2003, 2004 and 2005.

The particle size reduction procedure was the same as that previously described in the Chapter 3. All barley samples were coarsely dry-rolled through a 1.55 mm roller gap in a grain roller mill (Sven Grain Mill, Apollo Machine and Products Ltd., Saskatoon, Canada) in the College of Engineering, University of Saskathcewan.

In Situ Rumen Incubation

Animals and Diets

Three Holstein dry cows weighing approximately 670 kg were rumenly fistulated and housed individually in a box stall with bedded straw in the metabolism facilities at the University of Saskatchewan. The cows had ad libitum access to fresh water and were free to enter the exercise ground. The cows were fed twice daily at 0800 and 1600 h and received equal portions (7 kg at each feeding time) of total mixed ration, consisting of 56.8% barley silage, 10.2% alfalfa hay, 4.5% dehydrated alfalfa pellets, 21.6% standard dairy concentrate and 6.8% fresh cow concentrate on dry matter base (Appendix 5). The diet was introduced over a 10-day adaptation period. The animals used in the experiment were cared for according to the guidelines provided by Canadian Council on Animal Care (1993).

Rumen Incubation

Rumen degradation experiments were performed in two separate sets of in situ incubation trials following the nylon bag technique procedure described by Yu et al. (2003).

Trial 1: Rumen Degradability of DM, FA, PCA, NDF, ADF and ADL of Six Barley Varieties at 12 and 24 h Rumen Incubation

The first trial examed the rumen degradability of barley DM, NDF, ADF, ADL, FA and PCA of the six barley varieties at 12 and 24 h of rumen incubation. Coded nylon bags (Nitex 03-41/31 monofilament open mesh fabric, ScreenTec Corp., Mississauga, ON; 10 cm × 20 cm; pore size of 41 μm) were filled with 7 g of ground sample per bag. The ratio of sample weight to bag surface area was roughly 19 mg/cm^2. Three and four bags per sample were arranged for 12 and 24 h periods respectively to ensure that there was sufficient sample residue for chemical analysis. Bags were randomized and individual bags were placed in a large mesh bag (28 nylon bags per mesh bag) with a heavy bottle for the ballast and placed in the ventral sac of the rumen at 2100 pm for 24 h and at 0900 am for 12 h according to the 'gradual addition/all out' schedule. The trial was carried out in three runs. Following removal from the rumen, all the bags were washed together under cold tap water without detergent for six rinses with gentle agitation until the effluent remained clear. Subsequently, bags were dried in a forced-air oven at 55 °C for 48 h, then left in the open air to air equilibrate for three days. The residues were weighed and pooled together according to treatment and incubation time. DM was determined based on pooled residues. Pooled samples were ground in a Retsch ZM–1 grinder (Brinkmann Instruments Canada Ltd, Ontario) using a 1-mm screen. Samples were mixed and separated into two portions: one for fiber analysis; the other was reground through a 0.25 mm screen size for determining FA and PCA content following the same procedure in the Chapter 3.

Trial 2: In Situ Rumen Degradation Kinetics of Two Barley Varieties

Based on the results from Trial 1, two barley varieties were selected for further analysis of rumen degradation kinetics in detail, which were CDC Dolly ("better") and McLeod ("poorer"), respectively. Trial 2 was performed for these two barley varieties to determine whether or not FA and PCA fit into the first-order digestion kinetics equation (Ørskov and McDonald 1979) and to compare the differences of the degradation characteristics for DM, FA and PCA. The two barley varieties were from three years (2003, 2004, 2005). Year was used as experimental replication. The incubation periods in Trial 2 were increased to eight incubations in the sequence of 72, 48, 24, 12, 8, 4, 2 and 0 h according to the 'gradual addition/all out' schedule (Appendix 6). To ensure enough residue left for further analysis, increasing bag numbers were arranged following the ascending incubation time which is listed in Appendix 6. Following incubation, all the bags (including 0 h bags) were washed and dried with the same procedure as Trial 1.

Rumen Degradation Kinetics

For Trial 1, the percentage of rumen indigestible residues of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h was calculated from the content of these components in original samples and in residues.

For Trial 2, the percentage of rumen indigestible residues of DM, FA and PCA at each incubation was calculated and fitted into the nonlinear model using the PROC NLIN procedure of SAS (2002) with iterative least squares regression (Gauss- Newton method) by following the first-order digestion kinetics equation (Ørskov and McDonald 1979) modified by Tamminga et al. (1990, 1994):

$$R(t) = U + (100 - S - U) \times e^{-K_d \times (t - T_0)} \quad (t > T_0)$$

As long as RSS meets the convergence criterion, the rumen degradation data is assumed to fit the Ørskov first-order kinetics model (Mustafa 1996). In the equation, R(t) means the amount of residue (%) of the original sample incubated after t hour. DM, FA and PCA were partitioned into three fractions based on the relative susceptibility to rumen degradation. Fraction S was defined as the instantly digestible or 'wash-out' fraction (S, %); fraction U corresponded to the undegradable fraction in the rumen (U, %); and fraction D was considered as the slowly degradable fraction (D, %), which was degraded exponentially in the rumen. T_0 (h) was lag time and K_d (% h^{-1}) was disappearance rate constant (K_d, % h^{-1}) were determined directly from this model, and fraction S was estimated from 0 h. The effective degradabilities (ED) for DM, FA and PCA were calculated as:

$$ED (\%) = S + D \times K_d/(K_p + K_d)$$

Where, K_p is the fractional passage rate and assumed to be 6% h^{-1} (Yu et al. 2003). The rumen undegraded proportions (RU) of DM, FA and PCA were determined as:

$$RU (\%) = U + D \times K_p/(K_p + K_d).$$

Where, S, U, D, K_p, K_d were defined as above.

Statistical Analysis

Analysis of variance (ANOVA) for rumen degradation parameters was performed using SAS (2002). Significance was declared at $P < 0.05$. Factorial treatment arrangement (6×2: six varieties and two incubation times) and CRD experimental design were used to describe the effects of barley variety and rumen incubation time on the rumen degradation parameters (residue percentages of DM, FA, PCA, NDF, ADF and ADL), using the Proc Mixed in SAS (2002). Means were compared by LSD test. Rumen degradation parameters (S, D, U, K_d, T_0, ED, RU) for DM, FA and PCA were compared between two barley varieties seleted based on Trial 1 results with T test using PROC TTest procedure in SAS (2002).

Results and Discussion

Trial 1: Rumen Degradability of DM, FA, PCA, NDF, ADF and ADL of Six Barley Varieties at 12 and 24 h of Rumen Incubations

Table 1 shows the effect of barley variety and/or rumen incubation time on the in situ rumen indigestible residues (%DM) of barley DM, FA, PCA, NDF, ADF and ADL as well as the interaction between the barley variety and rumen incubation time.

Effects of variety ($P<0.05$) were observed on the rumen indigestible residues of DM, FA, PCA, NDF and ADF, but not ADL residues ($P=0.14$). Rumen incubation time had a significant effect ($P<0.05$) on the rumen indigestible residues of barley DM, FA, ADF and ADL. The higher the indigestible residue percentage left in the rumen, the lower the digestibility of the grain. The interaction effect between barley variety and rumen incubation time on the rumen indigestible residues was not observed.

Table 1. Rumen degradability of DM, FA, PCA, NDF, ADF and ADL of six barley varieties at 12 and 24 h of rumen incubations

		In situ rumen indigestible residue at 12 and 24 h (%)					
		DM	NDF	ADF	ADL	FA	PCA
Variety	AC Metcalfe	46.3 ^{abc}	61.3 ^{a}	85.2 ^{d}	87.1	60.3 ^{cd}	71.7 ^{b}
	CDC Cowboy	45.4 ^{bc}	64.4 ^{a}	89.9 ^{ab}	91.3	64.1 ^{b}	74.2 ^{b}
	CDC Dolly	44.1 ^{c}	61.9 ^{a}	86.6 ^{cd}	87.0	59.9 ^{cd}	72.1 ^{b}
	CDC Helgason	49.4 ^{a}	57.8 ^{b}	88.8 ^{bc}	89.4	59.7 ^{d}	71.9 ^{b}
	CDC Trey	47.6 ^{abc}	57.7 ^{b}	88.4 ^{bc}	89.4	72.3 ^{a}	80.6 ^{a}
	McLeod	49.0 ^{ab}	62.8 ^{a}	92.3 ^{a}	89.7	63.6 ^{bc}	74.7 ^{b}
	SEM	1.31	1.17	1.03	1.28	1.30	1.54
Incub. Time	12 h	56.6 ^{a}	61.4	90.1 ^{a}	90.1 ^{a}	64.8 ^{a}	74.6
	24 h	37.4 ^{b}	60.4	87.0 ^{b}	87.8 ^{b}	61.8 ^{b}	73.8
	SEM	0.76	0.68	0.60	0.74	0.75	0.89
Statistical analysis		--P value--					
	Variety	< 0.05	< 0.05	< 0.05	0.14	< 0.05	< 0.05
	Time	< 0.05	0.35	< 0.05	< 0.05	< 0.05	0.55
	Variety×Time	0.88	0.71	0.74	0.97	0.61	0.33

$^{a, b, c, d}$ Different superscripts in the same column are significantly different ($P < 0.05$).

The average DM indigestible residue percentages at 12 and 24 h of rumen incubation were 56.6% and 37.4%, respectively, and were different ($P<0.05$) (Table 1). DM residues in the present study were higher than those of Yu et al. (2003) who observed that the indigestible residues of coarsely dry-rolled barley (Harrington and Valier) were approxmately 21% at 24 h. This discrepancy could have resulted from the different grain particle sizes, as the roller gap used in current experiment was larger (1.55 mm vs. 0.53 mm). CDC Dolly showed relatively low DM residues (44.1%) after rumen digestion, which indicated CDC Dolly has a high DM digestibility that is favorable for ruminants. In contrast, CDC Helgason was the poorest (49.4%) feed barley for ruminants.

The residue percentages of fiber in the forms of NDF, ADF and ADL were much higher than DM as the fiber is more recalcitrant to rumen digestion. NDF represents the total structural cell wall components including cellulose and hemicellulose as well as lignin, so rumen indigestible NDF residue was lower than ADF and ADL, and averaged 61.4% and 60.4% at 12 and 24 h, respectively. Feng et al. (1995) reported 63-68% total tract indigestible NDF for whole barley grain, while Beauchemin et al. (1999) found it was 53% for the whole barley grain indicating that a range of variation for NDF digestibility exists. The NDF residue difference between 12 and 24 h was not significant ($P>0.05$), which implies that most of NDF in barley grain was degraded within 12 h of rumen incubation. ADF contains principally cellulose and lignin, which is less digestible than NDF. Beauchemin et al. (2001) found that rumen indigestible ADF for stream-rolled barley was about 80% compared to 50-65% of indigestible NDF in steers. In the present study, ADF residue at 12 and 24 h averaged 90.1% and 87.0%, respectively. Rumen ADF residues were different among barley varieties and between the two rumen incubation time-points ($P<0.05$). Among the six varieties, McLeod showed higher ($P<0.05$) ADF residue than CDC Dolly and AC Metacalf. Therefore, AC Metcalfe and CDC Dolly were superior to McLeod in terms of feed barley quality. Although ADL is thought to be of low digestibility for animals, in the present study, roughly 10% of ADL was degraded in the rumen. Nelson (2001) also reported that 12.2% ADL was degradable when lambs were fed a coarsely dry-rolled barley based diet. However, no statistical difference among barley varieties was detected for ADL rumen residues. The original content of ADL in barley was quite low (about 1-2%). This is perhaps because the original content of ADL in barley was quite low (1.7 – 2.1 %DM). In practice, ADL digestibility of barley grain is seldom analyzed.

To date, no one has studied the digestibility of esterified FA and PCA in barley using either in vitro, in situ or in vivo methods. Researchers are interested in FA and PCA in forages, because forages contain greater amounts of FA and PCA. In the present study, barley variety was also found to have significant effect on FA and PCA rumen residues ($P<0.05$) with highest FA and PCA rumen indigestible residues in CDC Trey. Although CDC Trey showed only moderate content of hull, FA, PCA and fiber, it exhibited higher ($P<0.05$) rumen indigestible residues of FA and PCA than others. The reason for this is not clear. With exception to CDC Trey, there was no difference in PCA residues among the remaining five barley varieties. FA residues at 12 and 24 h were different ($P<0.05$), decreasing from 64.8% to 61.8% on average. The change for PCA resiude was not obvious ($P = 0.55$), decreasing from 74.7% to 73.8%. This implies that FA in barley continued to be degraded in the rumen after 12 h of rumen incubation, while the degradation of PCA came to a plateau after 12 h rumen incubation. After the same rumen incubation periods (12 and 24 h), levels of FA residues were lower than PCA, indicating that FA was degraded to a relatively greater extent than PCA. Others have observed that esterified FA was degraded more quickly and to a greater extent than esterified PCA in forages (e.g. cocksfoot, orchardgrass) (Canale et al. 1990; Grabber and Jung 1991; Bourquin et al. 1994). However, PCA is more related to lignin, so its degradability probably will not affect much the digestion of plant cell wall polysaccharides.

When all the degradation parameters were compared, CDC Dolly showed relatively lower rumen residues of DM, FA, PCA, ADF and ADL compared to the other barley varieties, while McLeod seemed to be more resistant to rumen degradation with relatively higher in rumen residues of DM, PCA, ADF and ADF. In combination with the physical and chemical information reported in the previous chapter, we have found that CDC Dolly is more promising as a feed barley grain while McLeod is relatively inferior. Therefore, these two barley varieties were selected for the next experiment for detailed comparison of in situ rumen digestion kinetics of DM, FA and PCA.

Trial 2: In Situ Rumen Degradation Kinetics of Ferulic Acid and ρ-Coumaric Acid in Two Barley Varieties

Degradation Characteristics of DM

The rumen DM degradation characteristics of CDC Dolly and McLeod are presented in Table 2. Figure 1 illustrates the degradation curve. General parameters of in situ DM degradation in barley were comparable to the results described by Yu et al. (2003) and Walker (2007). As expected, CDC Dolly provided more nutrients for ruminants than McLeod because of its higher ($P<0.05$) potential digestible fraction of DM (D, %DM) and lower ($P<0.05$) undegradable fraction of DM (U, %DM) ($P<0.05$) (82.8 vs. 79.3 %DM, 13.7 vs. 16.2 %DM) (Table 2). The main undegradable fraction of barley is the hull. CDC Dolly also contained less content of hull, FA, PCA and fiber than McLeod. So, it is reasonable that CDC Dolly showed higher D fraction and lower U fraction than McLeod. The slowly digestible fraction is nutritionally important for ruminants because it contributes to the growth of rumen microbes by providing starch as the energy source (Khorasani et al. 2000).

Table 2. In situ degradation kinetics of dry matter in CDC Dolly and McLeod

Parameter	Barley Variety		
	CDC Dolly	McLeod	P value
S (% DM)	3.5	4.6	0.06
D (% DM)	82.8	79.3	<0.05
U (% DM)	13.7	16.2	<0.05
Kd (% h^{-1})	10.8	8.1	0.18
T_0 (h)	0.95	0.52	0.26
EDDM (% DM)	56.2	49.8	0.11
RUDM (% DM)	43.8	50.2	0.11

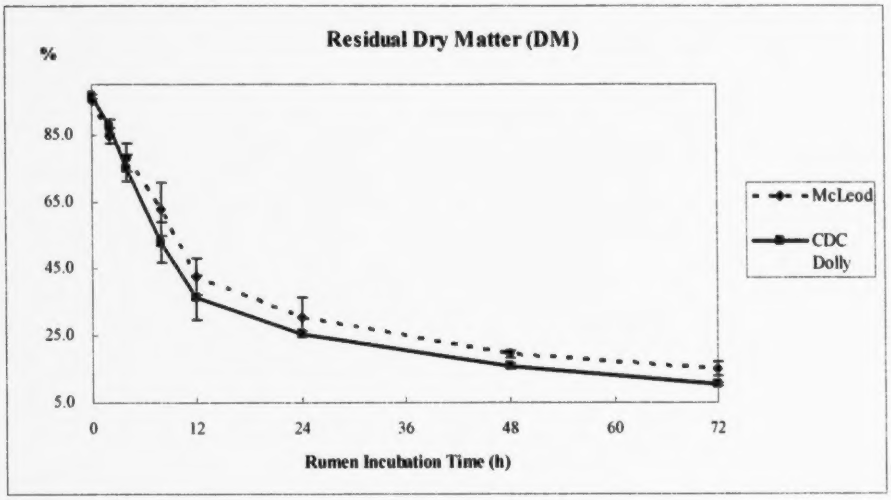


Figure 1. Dry matter residue of CDC Dolly and McLeod at various rumen incubations

CDC Dolly tended to exhibit a lower ($P = 0.06$) soluble portion of DM (S, %DM) (3.5 %DM) than McLeod (4.6 %DM) (Table 2), which could result from the difference of the intrinsic properties of barley grain (eg. the structural organization of protein, starch and the molecular structural chemical make-up) (McAllister et al. 1993; Walker 2007; Yu et al. 2008). Yu et al. (2003) observed large variation of soluble fractions between Valier and Harrington (1.5 vs. 11.6 %DM) which was owing to the difference of barley type (feed vs. malting). Rumen degradation rate (Kd, % h^{-1}) between CDC Dolly and McLeod did not differ ($P = 0.18$). Lag time (T_0, h) (0.95 h for CDC Dolly and 0.52 h for McLeod) was quite long compared to that of Yu et al. (2003) (0 - 0.2 h). This is likely due to a larger roller gap size (1.55 vs. 0.53 mm) used to process the grain in the present study. Lag time is a process of hydration and the initiation of rumen bacterial attachment (Mustafa 1996). Larger particle size could delay the process and lead to longer lag time. The effective degradation of DM (EDDM) between CDC Dolly and McLeod did not differ ($P = 0.11$), but CDC Dolly showed numerically higher EDDM (56.2 vs. 49.8 %DM), which indicated that CDC Dolly tended to be more extensively degraded in the rumen and of higher nutritional feed value than McLeod.

Rumen Degradation Characteristics of FA and PCA

The rumen degradation kinetics of FA and PCA were presented in Table 3 and Figure 2 for FA, and in Table 4 and Figure 3 for PCA. Canale et al. (1990) also employed the first-order digestion kinetics equation to describe the effect of alkali-treatment on in situ digestion of FA and PCA in orchardgrass and alfalfa. Bourquin et al. (1994) used the first-order exponential model to analyze in situ digestion profiles of DM, FA and PCA, and found that in situ degradation profiles of FA and PCA followed the patterns similar to that of DM.

Table 3 shows that there was no difference in the characteristics of FA degradation kinetics between

CDC Dolly and McLeod except for U (%FA) and RUFA (µg/g DM) where CDC Dolly tended to have lower U and RUFA than McLeod (P>0.05). This indicates that FA in CDC Dolly tended to have a higher extent of rumen degration than McLoed.

Table 3. In situ rumen degradation kinetics of ferulic acid in CDC Dolly and McLeod

Parameter	Barley Variety		P value
	CDC Dolly	McLeod	
S (% FA)	22.2	18.4	0.26
D (% FA)	44.4	41.9	0.22
U (% FA)	33.4	39.[illegible]	0.08
Kd (% h^{-1})	4.4	4.4	0.99
T_0 (h)	0	0	
EDFA (% FA)	40.9	36.1	0.19
EDFA (µg/g DM)	226.8	237.8	0.51
RUFA (% FA)	59.1	63.9	0.19
RUFA (µg/g DM)	328.3	424.8	0.06

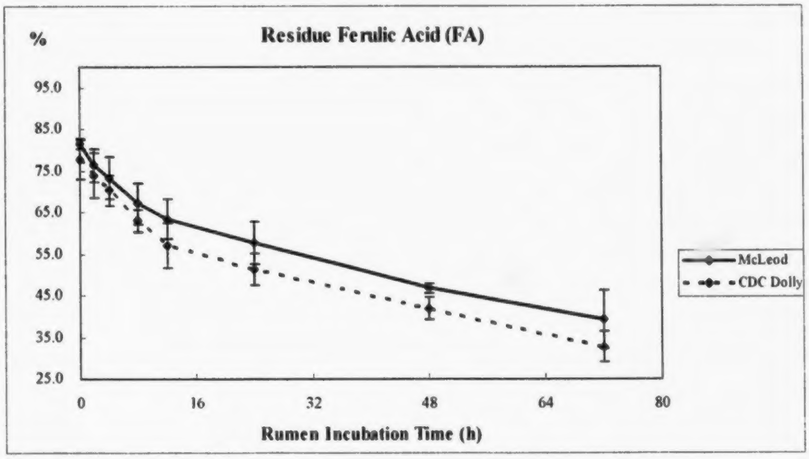


Figure 2. Ferulic acid disappearance of CDC Dolly and Mcleod at various rumen incubations

Table 4. shows in situ degradation kinetics of PCA in the two barley varieties. Significant differences were found in S (%PCA), U (%PCA), EDPCA (%PCA) and RUPCA (%PCA), which indicate that esterified PCA in CDC Dolly was more digestible than that in McLeod.

Table 4. In situ rumen degradation kinetics of para-coumaric acid in CDC Dolly and McLeod

Parameter	Barley Variety		
	CDC Dolly	McLeod	P value
S (% PCA)	17.1	10.9	<0.05
D (% PCA)	21.7	22.4	0.50
U (% PCA)	61.2	66.7	<0.05
Kd (% h^{-1})	9.7	9.2	0.85
T_0 (h)	0.81	0.97	0.72
EDPCA (%PCA)	30.3	24.4	<0.05
EDPCA (µg/g DM)	92.5	84.0	0.39
RUPCA (%PCA)	69.7	75.6	<0.05
RUPCA (µg/g DM)	213.0	261.2	0.06

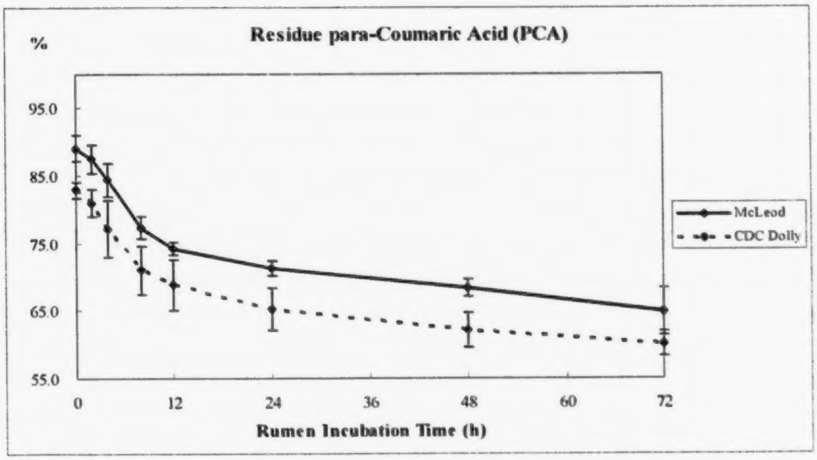


Figure 3. para-Coumaric acid disappearance of CDC Dolly and Mcleod at various rumen incubations

Comparing FA to PCA, FA had slower K_d (about 4.4% h^{-1}) than PCA (around 9.5% h^{-1}) which contrasted to the findings of Canale et al. (1990) for forages (7.8 % h^{-1} vs. 3.3 % h^{-1}). These authors also found that K_d for PCA increased with the maturity degree from prehead (3.3% h^{-1}) to head (7.5% h^{-1}) stage in forages. Bourquin et al. (1994) observed that animal diets and the degree of feed processing could affect

K_d values of FA and PCA in orchardgrass. The discrete lag time for FA was 0 h, while for PCA it was around 0.9 h. One possible explanation is that due to the difference in chemical structure, the esterified FA is relatively more easily accessible and degradable for rumen microbes and their enzymes than the esterified PCA. Another potential reason is that FA is both esterified to lignin and polysaccharides and is rich in both barley hull and aleurone layer, while PCA is mostly esterified to lignin in barley hull (Maillard and Berset 1995). Furthermore, aleurone layer is more likely to shatter during rolling than barley hull, so more FA could be washed out as the aleurone powder goes, which would likely contribute to 0 h lag time for FA. Canale et al. (1990) observed a shorter lag time for FA than for PCA in orchardgrass hay. EDFA (%FA) was higher than EDPCA (%PCA), which means the esterified FA was more digestible than the esterified PCA in the rumen (Canale et al. 1990; Grabber and Jung 1991; Bourquin et al. 1994; Agbagla-Dohnani et al. 2003a).

In general, most of the degradation kinetics parameters of DM, FA and PCA support CDC Dolly as a good feed grain.

Conclusions and Implications

Two consecutive in situ degradation experiments were carried out. The first trial was to determine and compare the differences in the rumen undegradable residues of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h of rumen incubation. The results show that barley variety had a significant effect ($P<0.05$) on rumen undegradable residue content of DM, FA, PCA, NDF and ADF either at 12 or 24 h of rumen incubation, but only a numerical effect on ADL. Among the six barley varieties, CDC Dolly demonstrated relatively lower content of rumen indigestible residues for most of parameters studied. In contrast, McLeod showed comparatively higher rumen residues and inferior digestibility. This information also implies that CDC Dolly could be a good candidate as a feed barley grain for ruminants.

Based on the first trial, two barley varieties (CDC Dolly and McLeod) were selected for further evaluation in the second experiment to study detailed degradation kinetics. This trial showed that the in situ rumen degradation parameters of FA and PCA fit the first-order digestion kinetics equation. This trial also showed that the esterified FA in barley grain was relatively more degradable than the esterified PCA. When degradation parameters for in situ degradation kinetics of DM, FA and PCA between CDC Dolly and McLeod were compared, no significant differences in EDDM and EDFA were seen, except for the significantly higher EDPCA of CDC Dolly than McLeod ($P<0.05$).

Acknowledgements

The authors would like to thank Saskatchewan Agriculture Development Fund (ADF, Project # 20040495) for financing this project and Saskatchewan Agriculture Development Fund (ADF) for Ministry of Agriculture Strategic Feed Research Chair Program.

PART I

Project 3: INVESTIGATION OF RELATIONSHIPS BETWEEN ORIGINAL CONTENT OF BARLEY HULL, HYDROXYCINNAMIC ACIDS, FIBER, PARTICLE SIZES IN VARIOUS BARLEY GRAIN AND IN SITU RUMEN DEGRADABILITIES

Project Details

Introduction

Barley is the principle energy grain for ruminants in western Canada. It contains moderately higher protein than corn, which could save cattle feeders from supplementing with other protein feeds. However, barley grain comprises proportionally lower content of starch than corn (Walker 2007) and shows relatively less digestible energy available for animals due to the presence and dilution effect of barley hull (NRC 2001). The inferiority of barley in terms of energy density is mainly due to the existence of the fibrous barley hull (about 13% of the kernel) (Evers et al. 1999). As is widely known, fiber playes an important role in affecting feed digestibility and energy density.

Other chemical components such as hydroxycinnamic acids are also reported to impose adverse effects on the digestibility of plant cell walls for ruminants (Canale et al. 1990; Jung and Deetz 1993; Grabber et al. 2004; Yu et al. 2005a). Barley grain contains two major low molecular weight hydroxycinnamic acids: FA (ferulic acid) and PCA (ρ-coumaric acid) (Nordkvist et al. 1984; Hernanz et al. 2001), which are chiefly concentrated in the cell walls of barley bran (Nordkvist et al. 1984; Sancho et al. 2001). FA is esterified to both plant cell wall polysaccharides and lignin, and is also etherified to lignin (Gubler et al. 1985; Sun et al. 2002). The extensive cross-linkages of FA between polysaccharides and lignin, and among cell wall polysaccharides form steric obstacles to protect plant cell wall polysaccharides from hydrolysis and attack by rumen microorganisms, thus reducing the digestibility of the plant in the rumen (Jung and Deetz 1993; Iiyama and Lam 2001; Moore and Jung 2001; Sun et al. 2002). PCA is chiefly esterified and etherified to cell wall lignin but seldom linked to polysaccharides, so PCA is not directly related to the digestibility of cell wall polysaccharides (Sun et al. 2002; Grabber et al. 2004).

It is common to coarsely dry-roll barley grain before feeding to cattle feedlots as dry-rolling helps to breach the rough barley hull, reduce the grain particle size, and improve its digestibility for ruminants (Mathison 1996). Particle size reduction is related to the intrinsic composition and structure of the grain. Processing barley has a positive effect on DM digestibility (Bradshaw et al. 1996). Nonetheless, excesively small particle size will not benefit the animal because rapid fermention of starch in the rumen will predispose cattle to greater incidences of disease and digestive problems. Therefore, maintenance of large particle size after the mechanical process is a preferred characteristic for a good feed barley grain.

Objectives

The objectives of this study were to investigate the relationship between the hull, chemical composition, different mean/median particle size of various barley varieties, and the in situ rumen nutrient digestibility at 12 and 24 h of incubation.

Material and Methods

Barley Samples

Six barley varieties from three consecutive years (2003, 2004, 2005) were used and included AC Metcalfe, CDC Dolly, CDC Helgason, CDC Trey, CDC Cowboy and McLeod.

Chemical Analysis

Original content of barley hull, FA, PCA, ratio of PCA/FA, total of PCA+FA, NDF, ADF, ADL, hemicellulose, cellulose, mean/median particle size, and residue content of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h of rumen incubation were analyzed and reported in the previous chapters 3 and 4.

Statistical Analysis

SAS (2002) procedure "PROC CORR" was used to examine the correlations among the variables of the barley hull, FA, PCA, NDF, ADF, ADL, and mean/median particle size, and residue content of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h of rumen incubation.

In order to develop prediction equations to show the effects of hull, FA, PCA and mean/median particle size on rumen undegradable residues of DM, NDF, ADF and ADL at 12 and 24 h of rumen incubation, muti-regression analysis was carried out using the "PROC REG" procedure of SAS (2002) with a model as follows:

$Y = \text{Hull} + \text{FA} + \text{PCA} + \text{PCA/FA} + \text{Mean} + \text{Median}$

Where, Y = Rumen degradability of NDF, ADF, ADL at 12 and 24 h of rumen incubation.

The model selection used a stepwise option. All variables left in the final prediction model are significant at the 0.05 level.

Results and Discussion

Correlation Analysis between Original Content of Hull, FA, PCA, Fiber, Particle Sizes and Rumen Indigestible Residues at 12 and 24 h of Rumen Incubation

The Effects of Barley Hull

The correlation analysis results are presented in Table 1. As expected, barley hull content was highly correlated to the content of NDF, ADF, ADL, hemicellulose and cellulose ($P<0.001$). It is widely recognized that the hull is the major contributor of fiber in barley grain. NRC (2001) reported that barley seed contains 20.8% NDF, 7.2% ADF and 1.9% ADL, while the hull representing about 13% of the total kernel weight is comprised of 72-79% NDF, 35-41% ADF and 7.2-8.4% ADL (Grove et al. 2003), which means that more than 45% NDF, 65% ADF and 50% ADL are from the hull. As such, the hull is of little nutritional value for monogastric animals because they cannot synthesize cellulolytic enzymes to digest fiber. Although ruminants are well-known for their capacity to utilize plant fiber, the digestibility of barley hull is as low as 32%, with only 14% disappearance for NDF (Grove et al. 2003). Therefore, a high percentage of barley hull will definitely lead to lower fiber digestion and lower digestible energy in barley. As seen in Table 1, in situ rumen indigestible residues of NDF and ADF were significantly correlated to barley hull content, which indicates that the higher hull content ($P<0.05$) results in lower NDF and ADF rumen digestibility.

However, the DM digestibility at both 12 and 24 h of rumen incubation was not significantly correlated with hull content ($P>0.05$) with low R value of 0.33 and 0.27, respectively. This is in contrast to the findings of Grove (2003). Some other factors may have exerted an effect on the DM digestibility rather than the barley hull. Although Grove (2003) observed that barley DM digestibility varied significantly with barley hull content, the author did not examine the effect of starch which was also significantly different among varieties. Barley hull had no significant ($P>0.05$) correlation with rumen residue content of FA, PCA and ADL. This indicates that not only the barley hull but also other factors, such as grain processing, content or structural matrix of starch and protein may play a role in determining rumen degradation of FA, PCA and ADL.

In general, the hull content was highly correlated to the content of FA, NDF, ADF, ADL, hemicellulose and cellulose in barley grain ($P<0.05$). Barley hull showed a significantly positive effect on rumen indigestible residue of NDF at 12 and 24 h, and ADF at 12 h ($P<0.05$). Therefore, among the hulled barley, the variety with less hull content would be preferred for ruminant feed.

Table 1. Correlation analysis for all parameters (original content of barley hull, FA, PCA, ration, total, NDF, ADF, ADL, hemicellulose, cellulose, mean/median particle size, and rumen in situ indigestible residues of DM, NDF, ADF, ADL, FA and PCA at 12 and 24 h of rumen incubation)

R value	Hull	FA	PCA	PCA/FA	PCA+FA	NDF	ADF	ADL	Hemi	Cell	Mean	Median
Original chemical and physical profiles in barley grain												
Hull	1.00	-	-	-	-	-	-	-	-	-	-	-
FA	0.57*	1.00	-	-	-	-	-	-	-	-	-	-
PCA	0.42	0.82***	1.00	-	-	-	-	-	-	-	-	-
PCA/FA	0.08	0.25	0.75***	1.00	-	-	-	-	-	-	-	-
PCA+FA	0.53*	0.96***	0.94***	0.49*	1.00	-	-	-	-	-	-	-
NDF	0.95***	0.76***	0.60**	0.15	0.72***	1.00	-	-	-	-	-	-
ADF	0.87***	0.71***	0.57*	0.15	0.68**	0.93***	1.00	-	-	-	-	-
ADL	0.84***	0.78***	0.64**	0.19	0.75***	0.91***	0.79***	1.00	-	-	-	-
Hemi	0.94***	0.75***	0.58*	0.15	0.71***	0.98***	0.85***	0.93***	1.00	-	-	-
Cell	0.78***	0.60**	0.47*	0.13	0.57*	0.82***	0.97***	0.61**	0.71***	1.00	-	-
Mean	0.31	0.70***	0.81***	0.57*	0.79***	0.45	0.35	0.49*	0.47*	0.25	1.00	-
Median	0.19	0.67**	0.79***	0.57*	0.76***	0.35	0.26	0.39	0.37	0.18	0.98***	1.00

* for P<0.05, ** for P<0.01, *** for P<0.001; Hemi = Hemicellulose, Cell = Cellulose, mean = mean particle size, median = median particle size

Table 1. (Cont'd)

R value	Hull	FA	PCA	PCA/FA	PCA+FA	NDF	ADF	ADL	Hemi	Cell	Mean	Median
Rumen in situ indigestible residue												
DM12	0.33	0.59**	0.42	0.04	0.54*	0.41	0.38	0.35	0.40	0.35	0.48*	0.50*
DM24	0.27	0.66**	0.57*	0.22	0.65**	0.38	0.26	0.40	0.41	0.17	0.60**	0.63**
NDF12	0.55*	0.34	0.18	-0.09	0.28	0.55*	0.48*	0.62**	0.55*	0.36	0.14	0.04
NDF24	0.63**	0.54**	0.67**	0.46	0.66**	0.67**	0.61**	0.75***	0.67**	0.49*	0.43	0.48*
ADF12	0.58*	0.33	0.12	-0.16	0.25	0.55*	0.64**	0.40	0.48*	0.66**	0.10	0.06
ADF24	0.46	0.49*	0.36	0.08	0.45	0.54*	0.67**	0.40	0.45	0.70***	0.33	0.30
ADL12	0.41	0.25	0.16	-0.03	0.22	0.42	0.44	0.49*	0.39	0.37	0.13	0.04
ADL24	0.36	0.11	-0.01	-0.09	0.06	0.32	0.39	0.31	0.28	0.37	0.09	0.07
FA12	0.35	-0.07	-0.11	-0.09	-0.09	0.22	0.22	0.18	0.21	0.22	-0.18	-0.21
FA24	0.33	0.13	0.16	0.10	0.15	0.27	0.31	0.32	0.24	0.28	0.01	-0.03
PCA12	0.28	-0.03	0.07	0.15	0.01	0.24	0.14	0.13	0.27	0.13	-0.10	-0.14
PCA24	0.26	0.04	0.12	0.14	0.08	0.28	0.31	0.31	0.25	0.28	-0.06	-0.11

* for $P<0.05$, ** for $P<0.01$, *** for $P<0.001$; Hemi = Hemicellulose, Cell = Cellulose, mean = mean particle size, median = median particle size

The Effects of FA and PCA, Ratio of PCA/FA and total of FA+PCA

FA and PCA are both esterified and etherified to plant cell wall components. Rumen microorganisms can produce phenolic acid esterases to ultimately break down the ester bonds, while the ether linkages are difficult to cleave in the rumen anaerobic environment (McSweeney et al. 1994; Jung and Allen 1995). Therefore, the esterified FA and PCA of rumen degradability were investigated in the present study.

FA, PCA and FA+PCA were highly correlated to the content of NDF, ADF, ADL, hemicellulose and cellulose ($P<0.05$). This is due to their close relationship with cell wall components. FA and PCA are mainly (about 80%) contained in the cell walls of barley bran (hull, pericarp, testa and aleurone layer) (Nordkvist et al. 1984; Hernanz et al. 2001; Chakraverty et al. 2003). However, the relationship between barley hull content and PCA was not significant ($P>0.05$), but was significant for FA ($P<0.05$). This could be explained by the different bonding models between FA and PCA in plant cell walls. PCA is heavily esterified and etherified to lignin, and seldom linked to cell wall polysaccharides, while FA is esterified to both lignin and polysaccharides, etherified to lignin, and forms cross-linkages between polysaccharides and lignin, and among polysaccharides (Iiyama et al. 1990; Lam and Tiyama 2000; Sun et al. 2002). Therefore, PCA is more related to the total lignin content in barley seed instead of the hull content. As can be found in Table 1, the correlation between PCA and ADL ($P<0.01$) is relatively stronger than that between PCA and NDF, ADF, hemicellulose, cellulose ($P<0.05$).

Interestingly, FA and PCA were very strongly correlated to mean/median particle size obtained by coarse dry-rolling ($P<0.001$). Experiments on wheat showed that higher content of FA, PCA and cross-linkages between FA and cell wall polysaccharides improved wheat bran's mechanical properties of extensibility, elasticity and plasticity (Antoine et al. 2003; Greffeuille et al. 2007).

The present results show that FA had positive effects on rumen indigestible residues of DM at 12 and 24 h, NDF and ADF at 24 h ($P<0.01$), while PCA showed effects on rumen indigestible residues of DM and NDF at 24h ($P<0.05$), which means that FA and PCA in barley grain are associated with the digestibility of barley grain in the rumen. Research has shown that rumen digestibility of plant cell walls are improved by releasing esterified FA and PCA from plant cell walls (Hartley 1983; Canale et al. 1990; Akin et al. 1991) and by reducing FA cross-linking in the plant (Jung and Phillips 2008). A computer model showed that in vitro rumen digestibility of hemicellulose was negatively correlated to the esterified FA and PCA, with FA having a greater negative influence than PCA (Jung et al. 1991). Jung et al. (1994) also observed a negative correlation between the content of esterified FA and the degradation of lucern cell walls. Casler and Jung (2006) observed a negative effect of esterified FA on 24h NDF in vitro digestibility in forages (Smooth bromegrass and Reed canarygrass). Rodrigues (2007) observed that FA and PCA content in hay showed negative effects on in situ digestibility of NDF and on the degradation rate of DM, but positive effects on effective digestibility of DM (EDDM). Their explanation was that FA and PCA content did not limit the extent of grass digestion, and the positive relationship between FA, PCA content and EDDM indicated that the deposition of FA and PCA and other cell wall nutrients were simultaneous. However, the nutrient deposition mechanisms in barley grain may be different from that in grasses, because the structural cell walls in barley grain are not evenly distributed as in grasses. In addition, FA imposed more of an impact on rumen digestion of DM, ADF and ADL at 12 and 24 h than PCA. Other authors have also observed more inhibitory effects of FA than PCA (Rodrigues et al. 2007). This probably results from the differences in bonding models.

The degradation of barley grain is a complex process, which is under the control of many factors. At 12 h of rumen incubation, more grain DM was retained than at 24 h; consequently, the effects of FA and PCA were more confounded by other factors (e.g. starch, protein). This explains why more influences were seen on 24 h residues rather than 12 h residues. In addition, FA and PCA seem to have almost no relationship (P>0.05) with rumen indigestible residues of ADL, FA and PCA at 12 and 24 h, which means that original FA and PCA content in barley grain did not affect the digestibility of ADL, FA and PCA in the rumen. Jung and Casler (1991) observed the different effects of lignin and esterified FA and PCA on the in vitro dry matter digestibility (IVDMD) of Smooth bromegrass leaf and stem and concluded that cross-linkages among the cell wall components influenced cell wall digestibility to a greater degree than the concentration alone.

The ratio of PCA/FA did not exhibit noticeable effects on most items examined, with the exception to mean/median particle size. Based on the observations from grasses studies, Grabber et al. (2004) proposed that the ratio of PCA/FA indicates the degree of lignification of plant tissues. In our experiment, the total content of FA and PCA in barley grain was analyzed; nonetheless, the distribution of FA and PCA in the whole seed is actually not even, with barley bran dominating (about 80%) in overall content (Nordkvist et al. 1984; Hernanz et al. 2001). Even within the bran fractions, barley hull is far more heavily lignified than percicarp, testa and aleurone layer. Therefore, the ratio of PCA/FA probably is not a good indicator of lignification in barley grain as it is in forages. Accordingly, the ratio of PCA/FA did not show a high correlation with barley fiber content. As for FA+PCA, it showed the collective effects of FA and PCA.

Generally, FA and PCA were found to be highly correlated to barley fiber (NDF, ADF, ADL, hemicellulose and cellulose) (P<0.05). FA and PCA content in barley grain affected (P<0.05) mean/median barley particle size obtained after coarse dry-rolling. Both FA and PCA showed inhibitory effects on the digestibility of barley DM, NDF and/or ADF (P<0.05) in the rumen, with FA exhibiting more negative influences than PCA in general. Rumen digestibility of ADL, FA and PCA were not related to the original content of FA and PCA in barley grain. The ratio of PCA/FA was not related to most parameters. According to current information, barley varieties with less FA and PCA content would be a good candidate for feed barley.

The Effects of NDF, ADF, ADL, Hemicellulose and Cellulose

Fiber is notorious for its recalcitrant properties. It is not only resistant to rumen digestion, but also retards, and even inhibits the availability of other nutrients to animals. The negative effects of barley fiber have been studied extensively. Our results show that the fiber in the form of NDF, ADF, ADL, hemicellulose and cellulose were significantly positively related to rumen indigestible residue content of NDF and ADF at either 12 or 24 h of rumen incubation (P<0.05). Numerical effects were found between barley fiber content and rumen indigestible residues of DM and ADL at 12 and 24 h. Profound differences would probably appear when the content of fiber is accumulated to a certain high degree, as is always seen in grasses and straws which contain more fiber (ADF, NDF, ADL) than barley grain. Rumen digestibilities of FA and PCA at 12 and 24 h were not significantly affected by the fiber. Fiber also showed some effects on particle size reduction after coarse dry-rolling. ADL and hemicellulose were positively correlated to mean particle size (P<0.05). Bowman et al. (2001) observed a positive relationship between ADF and particle size. Baumberger et al. (1998) stated that lignin could interact with starch and enhance the starch film's mechanical properties, while hemicellulose was reported to increase the extensibility of plant cell walls (Whitney et al. 1999). Barley bran cell walls are rich in fiber, which may, therefore, contribute to the particle size reduction during mechanical processing. However, barley hull and particle size were not significantly related. One possible explanation could be that fiber from the bran layers of pericarp, testa and aleurone play a more important role in determining the grain's mechanical resistance, as happens in wheat grain (Antoine et al. 2003).

In general, barley content of NDF, ADF, ADL, hemicellulose and cellulose were related to the rumen digestibility of NDF, ADF and ADL. Nutritionally, a barley variety with low fiber content would be an ideal feed grain with moderate content of fiber a benefit to maintain larger particle size during mechanical process.

The Effects of Mean/Median Grain Particle Size

A good feed barley variety should have high nutrient content, good nutrient availability, slow rate of rumen starch fermentation and maintain large particle size after mechanical processing. Although the degradation rate for processed barley grain with different particle size could not be directly compared, the in situ rumen digestion study at 12 and 24 h in the present study shows that larger mean/median barley particle size resulted in increased rumen indigestible residues of DM ($P<0.05$). This implies that larger particle size reduced DM digestibility. Researchers at the Dairy Research and Technology Center in University of Alberta reported that barley particle sizes that were generated by grinding the barley through 2, 4 and 6 mm screens did not significantly affect effective dry matter digestibility (EDDM) and rumen degradation rate (K_d) of DM, but their results also showed a trend of decreasing EDDM with increasing grain particle size (Nikkhah et al. Undated). Further research is needed to disclose the relationship between particle size and barley digestibility in the rumen. No significant ($P>0.05$) effects were observed on the digestibility of NDF, ADF and ADL at 12 and 24 h. Boyles et al. (2000) stated that mechanical processing methods were employed to improve starch and protein digestibility of the grain; they further stated that the rolling method did not improve cellulose digestibility, a result also found in the present study. Mean/median particle size exerted negligible effects on FA and PCA digestion in the rumen.

In general, mean/median barley particle sizes obtained after coarse dry-rolling significantly reduced DM digestibility in the rumen at 12 and 24 h incubation, and showed no correlation effect with the digestibility of fiber, FA and PCA at 12 and 24 h rumen incubation.

Multi-regression Analysis between Hull, FA, PCA Content, Mean/median Particle Size and Rumen Indigestible Residues at 12 and 24 h of Rumen Incubation

The best models deduced from the stepwise multi-regression analysis are presented in Table 2. to illuminate the relationships between the in situ rumen indigestible residues of DM, NDF, ADF and ADL at 12 and 24 h and the original content of hull, FA, PCA, mean/median particle size.

Table 2. Multi-Regression analysis to find most important variables to predict rumen degradability using physiochemical characteristics: hull, FA, PCA, ratio, and mean/median particle size with tested multi-regression model as follows:
Model: Y (degradability) = Hull + FA+ PCA + PCA/FA ratio + Mean + Median

Predicted variables (Y)	Variable(s) selection [z] (Variables left in the model with P<0.05)	Prediction Equations Test model: Y=a+ b1*x1 + b2*x2+	Model R^2 value	RSD[y]	P value
DM12	Only FA left in the model	DM12 = 10.01 + 0.05 * FA	0.354	3.79	0.009
DM24	Only FA left in the model	DM24 = 10.89 + 0.04 * FA	0.439	2.84	0.003
NDF12	Only Hull left in the model	NDF12 = 27.17 + 3.38 * Hull	0.298	3.51	0.019
NDF24	Only Hull left in the model	NDF24 = 19.68 + 4.14 * Hull	0.392	3.49	0.005
ADF12	Only Hull left in the model	ADF12 = 54.71 + 3.50 * Hull	0.333	3.35	0.012
ADF24	Only FA left in the model	ADF24 = 64.12 + 0.04 * FA	0.236	3.90	0.041
ADL12	No variable met the 0.05 significant level for entry the model				
ADL24	No variable met the 0.05 significant level for entry the model				

[z] Model variable selection using multi-regression analysis with a stepwise option with variable selection: "slentry=0.05, slstay=0.05"

[y] RSD= Residue standard deviation. For other abbreviations, please see the abbreviation list in the text.

As described in the in situ rumen digestion experiments, FA showed a negative effect on DM digestibility in the rumen. The present regression analysis further shows that the original content of FA in barley grain accounted for 35.4% and 43.9% of the total variation in the rumen indigestible residues of DM at 12 and 24 h. However, this result contrasts to the observation of Rodrigues et al. (2007) on meadow hays. The discrepancy probably is originated from the different physical and chemical properties between grain and grasses. In barley grain, bran (possessing more than 80% of the total barley grain cell walls) is the protective layer for the inner vulnerable starchy endosperm and embryo. The presence of FA could enhance the resistance of the hull, pericarp, testa and aleurone layer to the digestion of rumen microorganisms and enzymes since FA is extensively cross-linked to cell wall polysaccharides and lignin (Jung and Deetz 1993; Grabber et al. 1998; Moore and Jung 2001).

Multi-regression analysis also shows barley hull was the most limiting factor for the in situ rumen digestion of NDF at 12 and 24 h with coefficients of determination (R^2) being 0.298 and 0.392, respectively ($P<0.05$). The hull is a large contributor to NDF content (about 45% of total NDF) in barley grain, so it is possible that higher hull content will lead to higher NDF residue in the rumen. The effects of FA and hull on DM and NDF residues increased when the incubation time proceeded from 12 to 24 h.

As for the in situ residues of ADF, hull content was the main source of variation at 12 h, but at 24 h, FA replaced hull and became the most restraining effect. The reason for this is unclear. Barley hull ADF represents 70% of total barley ADF content, so the hull is supposed to be a major effect on the ADF digestibility as is happened to NDF.

The ADL content in the hull occupies 50% of the total ADL in barley grain. Although barley hull was the dominating factor for determining rumen indigestible residues of ADL at 12 and 24 h, the influence of barley hull on ADL residues in the rumen was not significant ($P>0.05$). This is probably because the lignin content in the barley grain is very low averaging 1.9% and the digestibility of lignin is also small, even negligible (less than 10% of total lignin), which collectively leads to statistically insensitive results.

In general, barley hull and FA were the two most effective factors in determining the in situ rumen indigestible residues of barley DM, NDF and ADF, among the parameters of the original content of hull, FA, PCA and mean/median particle size. Accordingly, hull and FA content in barley grain should be considered when estimating rumen digestibility of DM, NDF and ADF in barley grain.

Conclusions and Implications

All the parameters from the physical and chemical analysis were collected and examined for correlation and regression models. These parameters included original content of barley hull, FA, PCA, NDF, ADF, ADL, hemicellulose, cellulose and mean/median particle size after coarse dry-rolling in 18 barley samples, rumen indigestible residues of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h of rumen incubation.

The correlation study showed that barley hull was highly correlated to NDF, ADF, ADL, hemicellulose and cellulose content ($R>0.78$, $P<0.001$) and significantly correlated to FA ($R=0.57$, $P<0.05$) but not to PCA ($R=0.42$, $P>0.05$). Barley hull was also significantly and positively related to rumen indigestible residues of NDF and ADF at either 12 or 24 h, but not to rumen residues of ADL, FA and PCA at 12 and 24 h. FA, PCA or FA+PCA were highly and positively correlated to barley NDF, ADF, ADL, hemicellulose, cellulose, mean/median particle size, and rumen indigestible residues of DM, NDF and ADF at either 12 or 24 h ($P<0.05$), with FA being more effective than PCA. The ratio of PCA/FA was not significantly correlated to most of the parameters. The original content of FA and PCA was not significantly correlated to the indigestible residues of ADL, FA and PCA at 12 and 24 h. NDF, ADF, ADL, hemicellulose and cellulose were highly interrelated ($P<0.05$) and were significantly and positively correlated to rumen indigestibe residues of NDF and ADF at 12 and 24 h, but not the residues of DM, ADL, FA, and PCA at 12 and 24 h of rumen incubation. Mean/median particle size of barley grain positively influenced the rumen indigestible residue content of DM, but not others. The correlation analysis results implied that reduction of barley hull, FA and PCA content could increase the digestibility of barley grain in ruminants. Changing mean/median particle size of barley grain obtained after dry-rolling could contribute to the digestibility of DM in the rumen.

Multi-regression with model variable selection analysis revealed that FA was the factor most inhibiting to DM degradability of barley in the rumen, and was the most effective factor to predict DM digestibility, while hull was the most effective factor to determine NDF digestibility in the rumen. Both hull and FA affected ADF degradability in the rumen.

All the results indicate that breeding barley varieties with lower hull and FA content would result in higher digestibility, higher energy density and higher quality of feed barley.

Acknowledgements

The authors would like to thank Saskatchewan Agriculture Development Fund (ADF, Project # 20040495) for financing this project and Saskatchewan Agriculture Development Fund (ADF) for Ministry of Agriculture Strategic Feed Research Chair Program.

PART I: GENERAL CONCLUSIONS

Barley is the principle energy grain for ruminants in western Canada. There are many hulled barley varieties grown in western Canada. Due to the extensive usage of barley grain in cattle diets, it is of economic importance to screen a good feed barley to maximize animal production. A variety with low content of hull, FA, PCA, fiber, and maintaining large particle size after mechanical processing would be the ideal feed barley for ruminants. The basic objective of this study was to compare the physical and chemical differences among the six barley varieties in relation to rumen nutrient availability and find a relatively ideal barley variety.

In the first study, chemical and physical profiles of barley samples were analyzed. This study showed that the variety had significant impacts ($P<0.05$) on the chemical and physical characteristics of barley, with CDC Helgason and CDC Dolly consistently showing relatively lower content of barley hull, FA, PCA and fiber than McLeod and CDC Cowboy, while mean/median particle size was not different among CDC Cowboy, CDC Helgason, McLeod and CDC Dolly. The results show that CDC Helgason and CDC Dolly would be promising as good feed barley varieties for ruminants.

The second study was to assess the differences in the in situ rumen digestibility of DM, NDF, ADF, ADL, FA and PCA at 12 and 24 h of rumen incubation. Barley variety again showed effects on rumen digestibility and the results revealed that CDC Dolly consistently showed relatively better rumen digestibility of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h, with McLeod the worst.The combinated results of the first and the second study showed that CDC Dolly was of superior quality and McLeod was of inferior quality as ruminant feed grain.

The third study was to compare the differences in the rumen degradation kinetics of DM, FA and PCA in CDC Dolly and McLeod. The rumen indigestible residue data were fitted into the first-order kinetics of degradation model. CDC Dolly showed lower RUDM, RUFA, and RUPCA than McLeod. In general, CDC Dolly exhibited better digestibility of DM, FA and PCA than McLeod, which continues to indicate that CDC Dolly was of better quality as a feed barley grain for ruminants.

The last study examined the correlation and regression analysis between the data of the chemical and physical analysis and the in situ rumen digestibility at 12 and 24 h. Results show that the content of barley hull, FA, PCA, fiber and grain particle size all had negative effects on the rumen digestibility of barley grain, with FA having the most negative influence on barley grain's DM digestibility, and the hull being responsible for the inhibitory influence on the digestibility of NDF and ADF. FA affects barley grain's DM digestibility through its cross-linking with cell wall polysaccharides and lignin and works as a steric obstacle and shields the esterified polysaccharides from enzymatic hydrolysis in the rumen (Moore and Jung 2001).

The present studies show that CDC Dolly is the best candidate as a good feed barley grain for ruminants.

Future study could look at the quality of CDC Helgason which showed consistently low content of hull, fiber, FA, PCA and relatively large mean/median particle size. However, in the study on rumen in situ digestibility at 12 and 24 h, it did not perform well and failed to compete with CDC Dolly. If its low digestibility at 12 and 24 h resulted from the slow rate of digestion, CDC Helgason would also become a good candidate for feed barley.

Since FA and PCA showed negative effects on barley grain's digestibility, methods could be exploited to reduce their content in barley grain (mainly in the bran). This could be accomplished through barley breeding program or mechanical treatment of barley grain or feeding with enzymes and chemicals to improve nutrient availability.

Literature Cited (in Part I and Thesis One)

Agbagla-Dohnani, A., Cornu, A., Nozie, P., Besle, J. M., Dulphy, J. P., Doreau, M. and Grenet, E. 2003a Microbial degradation of rice and barley straws in the sheep rumen and the donkey caecum. J. Sci. Food Agric., 83: 383-394.

Agbagla-Dohnani, A., Nozie, P., Gaillard-Martine, B., Puard, M. and Doreau, M. 2003b. Effect of silica content on rice straw rumen degradation. J. Agric. Sci., 140: 183-192.

Akin, D. E. 1989. Histological and physical factors affecting digestibility of forages. Agron. J., 81: 17-24.

Akin, D. E., Borneman, W. S., Rigsby, L. and Martin, S. A. 1993. p-coumaroyl and feruloyl arabinoxylans from plant cell walls as substrates for rumen bacteria. Appl. Environ. Microbiol., 59: 644-652.

Akin, D. E., Hartley, R. D., Rigsby, L. L. and Morrison, W. H. 1991. Phenolic acids released from bermudagrass (*Cynodon dactylon*) by sequential sodium hydroxide treatment in relation to biodegradation of cell types. J. Sci. Food Agric., 58: 207-214.

Allerdings, E., Ralph, J., Schatz, P. F. and Gniechwitz, D. 2005. Isolation and structural identification of diarabinosyl 8-O-4-dehydrodiferulate from maize bran insoluble fibre. Phytochemistry, 66: 113-124.

Amariwiz, R. and Weidner, A. 2001. Content of phenolic acids in rye caryopses determined using DAD-HPLC method. J. Food Sci., 19: 201-205.

American National Standards Institute (ANSI). 1998. Method of determining and expressing particle size of chopped forage materials by screening. ASAE Standard. ANSI/ASAE S424.1.

American National Standards Institute (ANSI). 2003. Method of determining and expressing fineness of feed materials by sieving. ASAE Standard. ANSI/ASAE S319.3.

Anderson, V. and Schroeder, J. W. 1999. Feeding barley to dairy cattle. [Online] Available: http://www.ag.ndsu.edu/pubs/ansci/dairy/eb72w.htm [20 Aug. 2007].

Andersson, A. A. M., Elfverson, C., Andersson, R., Regne'r, S. and Aman, P. 1999. Chemical and physical characteristics of different barley samples. J. Sci. Food Agric., 79: 979-986.

Andreasen, M. F., Christensen, L. P., Meyer, A. S. and Hansen, A. 2000. Ferulic acid dehydrodimers in rye (*Secale cereale L.*). J. Cereal. Sci., 31: 303-307.

Antoine, C., Peyron, S., Mabille, F., Lapierre, C., Bouchet, B., Abecassis, J. and Rouau, X. 2003. Individual contribution of grain outer layers and their cell wall structure to the mechanical properties of wheat bran J. Agric. Food Chem., 51: 2026 -2033.

Association of Official Analytical Chemists (AOAC). 1990. Official methods of analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Balasta, M. L. F. C., Perez, C. M., Juliano, B. O., Villareal, C. P., Lott, J. N. A. and Roxas, D. B. 1989. Effects of silica level on some properties of *Oryza sativa* straw and hull. Can. J. Bot., 67: 2356-2363.

Bartolomé, B. and Gómez-Cordovées, C. 1999. Barley spent grain: release of hydroxycinnamic acids (ferulic and ρ-coumaric acids) by commercial enzyme preparations. J. Sci. Food Agric., 79: 435-439.

Bartolomé, B., Kroon, P. A., Faulds, C. B., Waldron, K. W. and Gilbert, H. J. 1997. An *Aspergillus niger* esterase (ferulic acid esterase III) and a recombinant *Pseudomonas fluorescens subsp. cellulosa* esterase (Xy1D) release a 5-5' ferulic dehydrodimer (diferulic acid) from barley and wheat cell walls. Appl. Environ. Microbiol.. 63: 208-212.

Baumberger, S., Lapierre, C. and Monties, B. 1998. Utilization of pine kraft lignin in starch composites: impact of structural heterogeneity. J. Agric. Food Chem., 46: 2234-2240.

Beauchemin, K. A., Rode, L. M. and Yang, W. Z. 1997. Effects of nonstructural carbohydrates and source of cereal grain in high concentrate diets of dairy cows. J. Dairy Sci., 80: 1640-1650

Beauchemin, K. A., Yang, W. Z. and Rode, L. M. 1999. Effects of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows. J. Dairy Sci., 82: 378-390.

Beauchemin, K. A., Yang, W. Z. and Rode, L. M. 2001. Effects of barley grain processing on the site and extent of digestion of beef feedlot finishing diets. J. Anim. Sci., 79: 1925-1936.

Beauchemin, K. A. 2002. Applying Nutritional Management to Rumen Health. [Online] Available:http://www.das.psu.edu/dairynutrition/documents/beauchemin.pdf [March 2008].

Beecher, B., Bowman, J., Martin, J. M., Bettge, A. D., Morris, C. F., Blake, T. K. and Giroux, M. J. 2002. Hordoindolines are associated with a major endosperm-texture QTL in Barley (*Hordeum vulgare*). Genome. 45: 584-591.

Bhatty, R. S. 1999. The potential of hull-less barley. Cereal Chem., 76: 589-599.

Blokker, P., Boelen, P., Broekman, R. and Rozema, J. 2006. The occurrence of ρ-coumaric acid and ferulic acid in fossil plant materials and their use as UV-proxy. Plant Ecology, 182: 197-207.

Borneman, W. S., Akin, D. E. and VanEseltine, W. P. 1986. Effect of phenolic monomers on rumen bacteria. Appl. Environ. Microbiol.. 52: 1331-1339.

Bourquin, L. D., Garleb, K. A., Merchen, N. R. and G. C. Fahey, J. 1990. Effects of intake and forage level on site and extent of digestion of plant cell wall monomeric components by sheep. J. Anim. Sci., 68: 2479-2495.

Bourquin, L. D., Titgemeyer, E. C., Milgen, J. V. and George C. Fahey, J. 1994. Forage level and particle size effects on Orchardgrass digestion by steers: II. Rumen digestion kinetics of cell wall components. J. Anim. Sci.. 72: 759-767.

Bowman, J. G. P., Blake, T. K., Surber, L. M. M., Habernicht, D. K. and Bockelman, H. 2001. Feed-quality variation in the barley core collection of the USDA national small grain collection. Crop Sci., 41: 863-870.

Boyles, S. L., Anderson, V. L. and Koch, K. B. 2000. Feeding barley to cattle. [Online] Available: http://beef.osu.edu/library/barley.html [1 Sep. 2007].

Bradshaw, W. L., Hinman, D. D., Bull, R. C., Everson, D. O. and Sorensen, S. J. 1996. Effects of barley variety and processing methods on feedlot steer performance and carcass characteristics. J. Anim. Sci., 74: 18-24.

Brett, C. T., Wende, G., Smith, A. C. and Waldron, K. W. 1999. Biosynthesis of cell-wall ferulate and diferulates. J. Sci. Food Agric.. 79: 421-424.

Briggs, D. E. 1998. Malts and malting. 1st ed. Blackie Academic, New York, NY.

Briggs, D. E., Boulton, C. A., Brookes, P. A. and Stevens, R. 2004. Brewing: science and practice. 1st ed. CRC Press LLC, Boca Raton. FL.

Broderick, H. M. and Vogel, E. H. 1977. The practical brewer: a manual for the brewing industry. 2nd ed. Master Brewers Association of the Americas, Mdison, WI.

Bunzel, M., Ralph, J., Funka, C. and Steinharta, H. 2005. Structural elucidation of new ferulic acid-containing phenolic dimers and trimers isolated from maize bran. Tetrahedron Lett., 46: 5845-5850.

Bunzel, M., Ralph, J., Kim, H., Lu, F., Ralph, S. A., Martia, H. M., Hatfield, R. D. and Steinhart, H. 2003. Sinapate dehydrodimers and sinapate-ferulate heterodimers in cereal dietary fiber. J. Agric. Food Chem.. 51: 1427-1434.

Bunzel, M., Ralph, J., Marita, J. M., atfield, R. D. and Steinhart, H. 2001. Diferulates as structural components in soluble and insoluble cereal dietary fibre. J. Sci. Food Agric., 81: 653-660.

Burritt, E. A., Bittner, A. S., Street, J. C. and Anderson, M. J. 1984. Correlations of phenolic acids and xylose content of cell wall with in vitro dry matter digestibility of three maturing. J. Dairy Sci., 67: 1209-1213.

Buxton, D. R. 1989. In vitro digestion kinetics of temperate perennial forage legume and gras systems. Crop Sci.. 29: 213-219.

Caldwell, K. S., Langridge, P. and Powell, W. 2004. Comparative sequence analysis of the region harboring the hardness locus in barley and its colinear region in rice. Plant Physiol.. 136: 3177-3190.

Camm, G. A. 2008. Grain hardness and slow dry matter disappearance rate in barley. MSc. Thesis. Department of Plant Sciences. Saskatoon. SK.

Campbell, G. M., Bunn, P. J., Webb, C. and Hook, S. C. W. 2001. On predicting roller milling performance. II. the breakage function. Powder Technol., 115: 243-255.

Canadian Food Inspection Agency (CFIA). 2003. Barley breeding report-portfolio for progress. [Online] Available: http://www.meristem.com/barleyRpt/BarleyRptWeb. pdf [1 Sep. 2007].

Canadian Food Inspection Agency (CFIA). 2007. List of varieties which are registered in Canada. [Online] Available: http://www.inspection.gc.ca/english/plaveg/ variet/lovric.pdf [1 Sep. 2007].

Canadian Wheat Board (CWB). 2004-2006. Variety surveys. [Online] Available: http:// www. cwb.ca/ public /en/farmers/surveys/variety/archive [20 Aug. 2007].

Canadian Wheat Board (CWB). 2007. CWB barley. [Online] Available: http://www. choicematters.gov.ab.ca/files/pdf/cwb_barley_0506.pdf [20 Aug. 2007].

Canale, C. J., Abrams, S. M., Varga, G. A. and Muller, L. D. 1990. Alkali-treated orchardgrass and alfalfa: Composition and in situ digestion of dry matter and cell wall components. J. Dairy Sci., 73: 2404-2412.

Casler, M. D. and Jung, H. G. 2006. Relationships of fibre, lignin, and phenolics to in vitro fibre digestibility in three perennial grasses. Anim. Feed Sci. Technol., 125: 151-161.

Chakraverty, A., Mujumdar, A. S., Raghavan, G. S. and Ramaswamy, H. S. 2003. Handbook of postharvest technology: cereals, fruits, vegetables, tea, and spices. Marcel Dekker, NY.

Chemey, D. J. R., Chemey, J. H., Patterson, J. A. and Axtell, J. D. 1992. In vitro rumen fiber digestion as influenced by phenolic-carbohydrate complexes released from sorghum cell walls. Anim. Feed Sci. Technol., 39: 79-93.

Chesson, A., Provan, G. J., Russell, W. R., Scobbie, L., Richardson, A. J. and Stewart, C. 1999. Hydroxycinnamic acids in the digestive tract of livestock and humans. J. Sci. Food Agric., 79: 373-378.

Chesson, A., Stewart, C. S. and Wallace, R. J. 1982. Influence of plant phenolic acids on growth and cellulolytic activity of rumen bacteria. Appl. Environ. Microbiol., 44: 597-603.

Collins, H. M., Logue, S. J., Jefferies, S. P., Stuart, I. M. and Barr, A. R. 1999. A study of the physical, biochemical and genetics factors influencing malt extract. The 9th Australian Barley Technical Symposium, Gosford, NSW.

Cruz, J. M., Domı´nguez, J. M., Domı´nguez, H. and Parajo, J. C. 2000. Preparation of fermentation media from agricultural wastes and their bioconversion into xylitol. Food Biotechnol., 14: 79-97.

Darlington, H. F., Rouste, J., Hoffmann, L., Halford, N. G., Shewry, P. R. and Simpson, D. J. 2001. Identification and molecular characterisation of hordoindolines from barley grain. Plant Mol. Biol., 47: 785-794.

Dehority, B. A. 2003. Rumen microbiology. Nottingham University Press, Nottingham, UK.

Dendy, D. A. V. and Dobraszczyk, B. J. 2001. Cereals and cereal products: Chemistry and technology. Aspen Publishers, Gaithersburg, MD.

Dhanoa, M. S. 1988. On the analysis of dacron bag data for lwo degradability feeds. Grass Forage Sci., 43: 441-444.

Dong, Y., Tsuzuki, E., Kamiunten, H., Lin, D., Terao, H., Matsuo, M. and Cheng, S. 2005. Molecular genetic analysis of QTLs for ferulic acid content in dried straw of rice (*Oryza sativa L.*). Biochem. Genet., 43: 25-34.

Edney, M.J. 1999. Canadian malting barley varieties for the future. Proceedings of the Canadian Barley Symposium. Winnipeg, Canada.

Edreva, A. M., Velikova, V. B. and Tsonev, T. D. 2007. Phenylamides in plants. Russ. J. Plant Physiol., 54: 287-301.

Ellnain-Wojtaszek, M. 1997. Phenolic acids from Ginkgo biloba L. Part I. Qualitative analysis of free and liberated by hydrolysis phenolic acids. Acta. Pol. Pharm., 54: 225-228.

Erekul, O., Ellmer, F., Yavas, I. and öncan-Sümer, F. 2007. Influence of variety and mineral N-fertilization on yield and brewing quality of spring barley (*Hordeum vulgare L.*) in Western Turkey. Arch. Agron. Soil. Sci., 53: 273-286

European Brewery Convention (EBC) Analysis Committee. 1998. Analytica, 4th ed.Verlag Hans Carl Getranke Fachverlag, Numberg, DE.

Evers, A. D., Blakeney, A. B. and O'Brien, L. 1999. Cereal structure and composition. Aust. J. Agric. Res., 50: 629-650.

Ewing, D. L., Johnson, D. E. and Rumper, W. V. 1986. Corn particle passage and size reduction in the rumen of beef steers. J. Anim. Sci., 63: 1509-1515.

Facchini, P. J., Hagel, J. and Zulak, K. G. 2002. Hydroxycinnamic acid amide metabolism: physiology and biochemistry. Can. J. Bot., 80: 577-589.

Fahey, G. C. and Jung, H. G. 1983. Lignin as a marker in digestion studies: a review. J. Anim. Sci., 57: 220-225.

Fairbairn, S. L., Patience, J. F., Classen, H. L. and Zijlstra, R. T. 1999. The energy content of barley fed to growing pigs: characterizing the nature of its variability and developing prediction equations for its estimation. J. Anim. Sci., 77: 1502-1512.

Fang, C. and Campbell, G. M. 2003. On predicting roller milling performance V: effect of moisture content on the particle size distribution from first break milling of wheat. J. Cereal. Sci., 37: 31-41.

FAOSTAT. 2005. Production and trade. [Online] Available: http://faostat.fao.org/site/ 291/default. aspx [10 Sep. 2007].

Faulds, C. B. and Williamson, G. 1991. The purification and characterization of 4-hydroxy-3-methoxycinnamic acid esterase from Streptomyces olivochromogenes. J. Gen. Microbiol., 137: 2339-2345.

Faulds, C. B. and Williams, G. 1999. The role of hydroxycinnamates in the plant cell wall. J. Sci. Food Agric., 79: 393-395.

Feng, P., Hunt, C. W., Pritchard, G. T. and Parish, S. M. 1995. Effect of barley variety and dietary barley content on digestive function in beef steers fed grass hay-based diets. J. Anim. Sci., 73: 3476-3484.

Field Crop Development Centre and Lacombe Research Centre. 2006. Cereal research report-variety descriptions. [Online] Available: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/webdoc4927/$FILE/06_BARLEYsection.pdf [1 Sep. 2007].

Fisher, D. S., Burns, J. C. and Pond, K. R. 1988. Estimation of mean and median particle size of ruminant digesta. J. Dairy Sci., 71: 518-524.

Fox, G., Sulman, M., Young, K. and Inkerman, A. 2001. Barley grain colour-objective measurement and biochemical studies. The 10th Australian Barley Technical Symposium. Canberra, ACT.

Fox, G. P., Kelly, A. M., Cakir, M., Bloustein, G., Poulsen, D. M. E., Inkerman, P. A. and Henry, R. J. 2006. Genetic impacts of the hull on barley grain quality. J. Inst. Brew., 112: 101 -107.

Gaines, R. L., Bechtel, D. B. and Pomeranz, Y. 1985. A microscopic study on the development of a layer in barley that causes hull-caryopsis adherence. Cereal Chem., 62: 35-40.

Gallant, D. J., Monredon, F. d., Bouchet, B., Tacon, P. and Delort-laval, J. 1991. Cytochemical study of intact and processed barley grain. Options Mediterraneennes, 20: 31-34.

Government of Alberta. 2007. Agronomic performance of general purpose barley. [Online] Available: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/crop 9480 [1 Sep. 2007].

Grabber, J. H., Hatfield, R. D. and Ralph, J. 1998. Diferulate cross-links impede the enzymatic degradation of non-lignified maize walls. J. Sci. Food Agric., 77: 193-200.

Grabber, J. H. and Jung, G. A. 1991. In-vitro disappearance of carbohydrates, phenolic acids, and lignin from parenchyma and sclerenchyma cell walls isolated from cocksfoot. J. Sci. Food Agric., 57: 315-323.

Grabber, J. H., Jung, G. A., Abrams, S. M. and Howard, D. B. 1992. Digestion kinetics of parenchyma and sclerenchyma cell walls isolated from orchardgrass and switchgrass. Crop Sci., 32: 806-810.

Grabber, J. H., Ralph, J. and Hatfield, R. D. 1998. Ferulate cross-links limit the enzymatic degradation of synthetically lignified primary walls of maize. J. Agric. Food Chem., 46: 2609 -2614.

Grabber, J. H., Ralph, J. and Hatfield, R. D. 2000. Cross-linking of maize walls by ferulate dimerization and incorporation into lignin. J. Agric. Food Chem., 48: 6106-6113.

Grabber, J. H., Ralph, J. and Hatfield, R. D. 2002. Model studies of ferulate-coniferyl alcohol cross-product formation in primary maize walls: Implications for lignification in grasses. J. Agric. Food Chem., 50: 6008-6016.

Grabber, J. H., Ralph, J., Lapierre, C. and Barriere, Y. 2004. Genetic and molecular basis of grass cell-wall degradability. I. lignin–cell wall matrix interactions. C. R. Biologies, 327: 455 -465.

Greffeuille, V., Mabillea, F., Roussetc, M., Ouryd, F. X., Abecassisa, J. and Lullien-Pellerin, V. 2007. Mechanical properties of outer layers from near-isogenic lines of common wheat differing in hardness. J. Cereal. Sci., 45: 227-235.

Grove, A. V., Hunt, C. W. and Hepton, J. 2003. Chemical composition and rumen fermentability of barley grain, hulls, and straw as affected by planting date, irrigation level, and variety. Professional Animal Scientist, 19: 273-280.

Gubler, F., Ashford, A. E., Bacic, A., Blakeney, A. B. and Stone, B. A. 1985. Release of ferulic acid from barley aleurone. II. Characterization of the feruloyl compounds released in response to GA3. Aust. J. Plant Physiol., 12: 307-317.

Guido, C. G., Piccaglia, R., Chiavari, G. and Vittorio, C. 1989. HPLC/ Electrochemical detection of lignin phenolics from wheat straw by direct injection of nitrobenzene hydrolysates. J. Agric. Food. Chem., 37: 985-987.

Hartley, R. D. 1983. Degradation of cell walls of forages by sequential treatment with sodium hydroxide and a commercial cellulase preparation. J. Sci. Food Agric., 34: 29-36.

Henry, R. J. 1988. The carbohydrates of barley grain: a review. J. Inst. Brew., 94: 71-78.

Hernanz, D., Nunez, V., Sancho, A. I., Faulds, C. B., Williamson, G., Bartolome, B. and Gomez-Cordoves, C. 2001. Hydroxycinnamic acids and ferulic acid dehydrodimers in barley and processed barley. J. Agric. Food Chem., 49: 4884-4888.

Holtekjolen, A. K., Kinitz, C. and Knutsen, S. H. 2006. Flavanol and bound phenolic acid content in different barley varieties. J. Agric. Food Chem., 54: 2253-2260.

Hoover, W. H. 1986. Chemical factors involved in rumen fiber digestion. J. Dairy Sci., 69: 2755-2766.

Hough, J. S. 1991. The biotechnology of malting and brewing. Cambridge University Press, New York, NY.

Briggs, D. E. and Hough, J. S. 1981. Malting and brewing science. Chapman and Hall, London, UK.

Ibrahim, A. F. 1971. A modified method for the determination of hull content of barley seeds. Savremena Poljoprivreda. 19: 33-38.

Iiyama, K. and Lam, T. B. T. 2001. Structural characteristics of cell walls of forage grasses-Their nutritional evaluation for ruminants-Review. Asian-Aust. J. Anim. Sci., 14: 862 -879.

Iiyama, K., Lam, T. B. T. and Stone, B. A. 1990. Phenolic acid bridges between polysaccharides and lignin in wheat internodes. Phytochemistry, 29: 733-737.

Iiyama, K., Lam, T. B. T. and Stone, B. A. 1994. Covalent cross-links in the cell wall. Plant Physiol., 104: 315-320.

Ikegawa, T., Mayama, S., Nakayashiki, H. and Kato, H. 1996. Accumulation of diferulic acid during the hypersensitive response of oat leaves to *Puccinia coronata f. sp. avenae* and its role in the resistance of oat tissues to cell wall degrading enzymes. Physiol. Mol. Plant. Pathol., 48: 245 -255.

Izydorczyk, M. S., Dexter, J. E., Desjardins, R. G., Rossnagel, B. G., Lagasse, S. L. and Hatcher, D. W. 2003. Roller milling of Canadian hull-less barley: optimization of roller milling conditions and composition of mill streams. Cereal Chem., 80: 637-644.

Izydorczyk, M. S., Lazaridou, A., Chornick, T. and Dushnicky, L. 2005. Molecular structure and degradation patterns of endosperm cell walls from barley differing in hardness and beta-glucan and protein content. The 18th North American Barley Researchers Workshop. Red Deer, AB.

Jung, H. G. and Allen, M. S. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci., 73: 2774-2790.

Jung, H. G. and Casler, M. D. 1991. Relationship of lignin and esterified phenolics to fermentation of smooth bromegrass fibre. Anim. Feed Sci. Technol., 32: 63-68.

Jung, H. G. and Deetz, D. A. 1993. Cell wall lignification and degradability. Page 315-346 *in* H.G. Jung et al., eds. Forage cell wall structure and digestibility. ASA-CSSA-SSSA, Madison, WI.

Jung, H. G. and Phillips, R,L. 2008. Reduced ferulate crosslink concentration is associated with improved fiber digestibility of corn stover at silage maturity. ADSA-ASAS annual conference, Indianapolis. Indiana. USA. July 7-11.

Jung, H. G., Smith, R. R. and Endres, C. S. 1994. Cell wall composition and degradability of stem tissue from lucerne divergently selected for lignin and in vitro dry matter disappearance. Grass Forage Sci., 49: 295-304.

Jung, H. G. and Vogel., K. P. 1992. Lignification of switchgrass (*Panicum virgatum*) and big bluestem (*Andropogon gerardii*) plant parts during maturation and its effect on fiber degradability. J. Sci. Food Agric.. 59: 169-176.

Jung, H. J. G., Ralph, J. and Hatfield., R. D. 1991. Degradability of phenolic acid- hemicellulose esters: a model system. J. Sci. Food Agric., 56: 469-478.

Khorasani, G. R., Helm, J. and Kennelly, J. J. 2000. In situ rumen degradation characteristics of sixty cultivars of barley grain. Can. J. Anim. Sci., 80: 691-701.

Kleber, W. and Franke, G. 1959. Eiweißgehalt und spelzenanteil von gersten. In: Braugersten Jahrbuch, 95 pp.

Koenig, K. M., Beauchemin, K. A. and Rode, L. M. 2003. Effect of grain processing and silage on microbial protein synthesis and nutrient digestibility in beef cattle fed barley-based diets. J. Anim. Sci., 81: 1057-1067.

Kononoff, P. J. 2005. Understanding effective fiber in rations for dairy cattle. [Online] Available: http://www.ianrpubs.unl.edu/epublic/live/g1587/build/g1587.pdf? [1 Sep. 2007].

Kroon, A. and Williamson, G. 1996. Release of ferulic acid from sugar-beet pulp by using arabinanse, arabinofuranosidase and an esterase from *Aspergillus niger*. Biotecholo. App. Biochem., 23: 263-267.

Kroon, P. A. and Williamson, G. 1999. Hydroxycinnamates in plants and food: current and future perspectives. J. Sci. Food Agric., 79: 355-336.

Kubán, P., Sterbová, D. and Kubán, V. 2006. Separation of phenolic acids by capillary electrophoresis with indirect contactless conductometric detection. Electrophoresis, 27: 1368 -1375.

Kulp, K. and Ponte, J. G. Jr. 2000. Handbook of cereal science and technology. 2nd ed. Marcel Dekker. New York. NY.

Lam, T. B. T., Iiyama, K. and Stone, B. A. 1992b. Changes in phenolic acids from internode walls of wheat and phalaris during maturation. Phytochemistry, 31: 2655-2658.

Lam, T. B. T., Iiyama, K. and Stone, B. A. 1992a. Cinnamic acid bridges between cell wall polymers in wheat and phalaris internodes. Phytochemistry, 31: 1179-1183.

Lam, T. B. T. and Tiyama, K. 2000. Characteristics of senescent straw cell walls of dwarf, semidwarf, and normal strains of rice *(Oryza sativa)* plants. J. Wood Sci., 46: 376-380.

Liang, Y., Chen, Q., Liu, Q., Zhang, W. and Ding, R. 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare L.*). J. Plant Physiol., 160: 1157-1164.

Lu, F. and Ralph, J. 1999. Detection and determination of p-coumaroylated units in lignins. J. Agric. Food Chem.. 47: 1988-1992.

Luff. 1898. über eine einfache methode zur bestimmung des spelzenanateils der gerste. Z.f.d. gesamte Brauwesen. 21: 485-487.

Maillard, M. N. and Berset, C. 1995. Evolution of antioxidant activity during kilning: Role of insoluble bound phenolic acids of barley and malt. J. Agric. Food Chem., 43: 1789-1793.

Manitoba Competitiveness Training and Trade. Undated. Barley. [Online] Available: http://www.gov.mb.ca/trade/globaltrade/agrifood/commodity/barley.html [20 Aug. 2007].

Mathew, S. and Abraham, T. E. 2004. Ferulic acid: an antioxidant found naturally in plant cell walls and feruloyl esterases involved in its release and their applications. Crit. Rev. Biotechnol., 24: 59-83.

Mathison, G. W. 1996. Effects of processing on the utilization of grain by cattle. Anim. Feed Sci. Technol., 58: 113-125.

Mathison, G. W. 2000. Alberta feedlot management guide. 2nd ed. Alberta Agriculture Food and Rural Development, Edmonton, AB.

Mazol, I., Glensk, M. and Cisowski, W. 2004. Polyphenolic compounds from Platycodon grandiflorum A. DC. Acta. Pol. Pharm., 61: 203-208.

McAllister, T. A. and Cheng, K. J. 1996. Microbial strategies in the rumen digestion of cereal grain. Anim. Feed Sci. Technol., 62: 29-36.

McAllister, T. A., Phillippe, R. C., Rode, L. M. and Cheng, K. J. 1993. Effect of the protein matrix on the digestion of cereal grain by rumen microorganisms. J. Anim. Sci., 71: 205-212.

McNeil, M., Albersheim, P., Taiz, L. and Jones, R. L. 1975. The structure of plant cell walls.VII. barley aleurone cells. Plant Physiol., 55: 64-68.

McSweeney, C. S., Dulieu, A., Katayama, Y. and Lowry, J. B. 1994. Solubilization of lignin by the rumen anaerobic fungus *Neocallimastix patriciarum*. Appl. Environ. Microbiol. 60: 2985-2989.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci., 80: 1463-1481.

Miyamoto, K., Ueda, J., Takeda, S., Ida, K., Hoson, T., Masuda, Y. and Kamisaka, S. 1994. Light-induced increase in the content of ferulic and diferulic acids in cell walls of *Avena coleoptiles*: its relationship to growth inhibition by light. Physiol. Plantarum, 92: 350-355.

Moore, J.E., Adesogan, A. T., Coleman, S. W. and Undersander, D. J. 2007. Predicting forage quality. Page 558 *in* R. F. Barnes et al., eds. Forages: the science of grassland agriculture. Blackwell Publishing, Ames, IA.

Moore, K. J. and Jung, H. J. G. 2001. Lignin and fiber digestion. J. Range Manage., 54: 420 -430.

Morrison, T. A., Jung, H. G., Buxton, D. R. and Hatfield, R. D. 1998. Cell wall composition of maize internodes of varying maturity. Crop Sci., 38: 455-460.

Mustafa, A. F. 1996. The nutritive value of high fiber canola meal for ruminants. PhD. Thesis. Department of Animal and Poultry Science, Saskatoon, SK.

Myton, K. E. and Fry, S. C. 1994. Intraprotoplasmic feruloylation of arabinoxylans in *Festuca arundinacea* cell cultures. Planta, 193: 326-330.

National Research Council (NRC). 2001. Nutrient requirements of dairy cattle. 7th revised edition. Washington. DC.

Nelson, M. L., Westberg, H. H. and Parish, S. M. 2001. Effects of tallow on the energy metabolism of wethers fed barley finishing diets. J. Anim. Sci., 79: 1892-1904.

Nikkhah, A., Khorasani, R., Kennelly, J. and Helm, J. Undated. Studies of barley feed quality. [Online] Available: http://www.westerndairyscience.com/html/U%20 of%20A%20articles/html/Barley1.html [1 Sep. 2007].

Noots, I., Derycke, V., Cornelis, K., Michiels, C., Delcour, J. A., Delrue, R., Keersmaeker, J. D. and Coppens, T. 2001. Degradation of starchy endosperm cell walls in nongerminating sterilized barley by fungi. J. Agric. Food Chem., 49: 975-981.

Nordkvist, E., Salomonssen, A. and Aman, P. 1984. Distribution of insoluble bound phenolic acids in barley grain. J. Sci. Food Agric.. 35: 657-661.

Olkku, J., Kotaviita, E., Salmenkallio-Marttila, M., Sweins, H. and Home, S. 2005. Connection between structure and quality of barley husk. J. Am. Soc. Brew. Chem., 63: 17-22.

Olkowski, A. A., Amarowicz, R., Yu, P., McKinnon, J. J. and Maenz, D. D. 2003. A rapid HPLC method for determination of major phenolic acids in plant material. Pol. J. Food Nutr. Sci., 12: 53-57.

Ondarza, M. B. d. 2006. Maintaining digestive health in dairy cattle. [Online] Available: http://www.milkproduction.com/Library/Articles/Maintaining_digestive. htm [20 Aug. 2007].

Ørskov, E. R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., Camb.. 92: 499-503.

Ørskov, E. R. and Ryle, M. 1990. Energy nutrition in ruminants. Elsevier Applied Scinece, London, U.K.

Ortega-Cerrilla, M. E., Finlayson, H. J. and Armstrong, D. G. 1999. Protection of starch in barley against rumen degradation by glutaraldehyde and formaldehyde as assessed by the dacron bag technique Anim. Feed Sci. Technol., 77: 83-90.

Oudgenoeg, G., Hilhorst, R., Piersma, S. R., Boeriu, C. G., Gruppen, H., Hessing, M., Voragen, A. G. J. and Laane, C. 2002. Peroxidase mediated cross-linking of a tyrosine-containing peptide with ferulic acid. J. Agric. Food Chem. 49: 2503-2510.

Owens, F. N., Secrist, D. S., Hill, W. J. and Gill, D. R. 1997. The effect of grain source and grain processing on performance of feedlot cattle. J. Anim. Sci., 75: 868-879.

Owens, F. N., Secrist, D. S., Hill, W. J. and Gill, D. R. 1998. Acidosis in cattle: a review. J. Anim. Sci., 76: 275-286.

Pasikatan, M. C., Steele, J. L., Milliken, G. A., Spillman, C. K. and Haque, E. 1999. Particle size distribution and sieving characteristics of first-break ground wheat. An ASAE meeting presentation. St. Joseph, MO.

Passarella, V. S. 2002. Grain weight and malting quality in barley as affected by brief periods of increased spike temperature under field conditions. Aust. J. Agric. Res., 53: 1219-1227

Pauly, T., Sporndly, R. and Uden, P. 1992. Rumen degradability in sacco of physically and chemically treated oat and barley grain. J. Sci. Food Agric., 58: 465-473.

Peng, Y., Liu, F. and Ye, J. 2005. Determination of phenolic acids and flavones in lonicera japonica thumb by capillary electrophoresis with electrochemical detection. Electroanalysis, 17: 356-362.

Piber, M. and Koehler, P. 2005. Identification of dehydro-ferulic acid-tyrosine in rye and wheat: Evidence for a covalent cross-link between arabinoxylans and proteins. J. Agric. Food Chem., 53: 5276-5284.

Pirjo, M., Pihlava, J. M. and Jarkko, H. M. 2005. Content of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. J. Agric. Food. Chem., 53: 8290-8295.

Pond, K. R., Tolley, E. A., Ellis, W. C. and Matis, J. H. 1984. A method for describing the weight distribution of particles from sieved forage. Pages 123-134 *in* P. M. Kennedy, ed. Techniques in particle size analysis of feed and digesta in ruminants. Canadian Society of Animal Science, Edmonton, AB.

Poppi, D. P., R. E. Hendricksen, and D. J. Minson. 1985. The relative resistance to escape of leaf and stem particles from the rumen of cattle and sheep. J. Agric. Sci. 105:9–14.

Priest, F. G. and Stewart, G. G. 2006. Handbook of brewing. 2nd ed. CRC/Taylor and Francis, Boca Raton, FL.

Psota, V., Vejražka, K., Faměra, O. and Hrčka, M. 2007. Relationship between grain hardness and malting quality of barley (*Hordeum vulgare L.*). J. Inst. Brew., 113: 80-86.

Ralph, J., Grabber, J. H. and Hatfield, R. D. 1995. Lignin-ferulate crosslinks in grasses: active incorporation of ferulate polysaccharide esters into ryegrass lignins. Carbohydr. Res, 275: 167 -178.

Ralph, J., Hatfield, R. D., Quideau, S., Helm, R. F. and Grabber, J. H. 1994a. Pathway of ρ-coumaric acid incorporation into maize lignin as revealed by NMR. J. Am. Chem. Soc., 116: 9448-9456.

Ralph, J. and Helm, R. F. 1993. Lignin/hydroxycinnamic acid polysaccharide complexes: synthetic models for regiochemical characterization. Pages 119–127 *in* H.G. Jung et al., eds. Forage cell wall structure and digestibility. ASA-CSSA-SSSA, Segoe Rd., Madison, WI.

Ralph, J., Quideau, S., Grabber, J. H. and Hatfield, R. D. 1994b. Identification and synthesis of new ferulic acid dehydrodimers present in grass cell-walls. J. Chem. Soc. Perkin Trans.: 3485 -3498.

Rémond, D., Cabrera-Estrada, J. I., Champion, M., Chauveau, B., Coudure, R. and Poncet, C. 2004. Effect of corn particle size on site and extent of starch digestion in lactating dairy cows. J. Dairy Sci., 87: 1389-1399.

Rodrigues, M. A. M., Guedes, C. M., Coneb, J. W., Gelder, A. H. v., Ferreira, L. M. M. and Sequeira, C. A. 2007. Effects of phenolic acid structures on meadow hay digestibility. Anim. Feed Sci. Technol., 136: 297-311.

Rouau, X., Cheynier, V., Surget, A., Gloux, D., Barron, C., Meudec, E., Louis- Montero, J. and Criton, M. 2003. A dehydrotrimer of ferulic acid from maize bran. Phytochemistry, 63: 899 -903.

Roumeliotis, S., Collins, H. M., Logue, S. J., Willsmore, K. L., Jefferies, S. P., and Barr, A. R. 2001. Implications of thin husk in barley. Proceedings of the 10th Australian Barley Technical Symposium.Canberra, Australia.

Rowell, R. M., Han, J. S. and Rowell, J. S. 2000. Characterization and factors effecting fiber properties. Natural Polymers and Agrofiber Composites. 115-134.

Russell, J. B. and Wilson., D. B. 1996. Why are rumen cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci., 79: 1503-1509.

Sancho, A. I., Bartolome´, B., Go´mez-Cordove´s, C., Williamson, G. and Faulds, C. B. 2001. Release of ferulic acid from cereal residues by barley enzymatic extracts. J. Cereal. Sci., 34: 173 -179.

SAS. 2002. User's guide: Statistics. 8th ed. SAS Institute Inc., Cary, NC.

Saskatchewan Wheat Pool. 2007. Seed grain. [Online] Available: http://www. swp.com/Seed/pdf/2007SeedGuide_feedgrain.pdf [25 Aug. 2007].

Saulnier, L., Crepeau, M. J., Lahaye, M. and Thibault, J. F. 1999. Isolation and structural determination of two 5,5'-diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross-linked by oxidatively coupled ferulates. Carbohydr. Res., 320: 82-92.

Saulnier, L., Vigouroux, J. and Thibault, J.-F. 1995. Isolation and partial characterization of feruloylated oligosaccharides from maize bran. Carbohydr. Res., 272: 241-253.

Savin, R. and Nicolas, M. E. 1996. Effects of short periods of drought and high temperature on grain growth and starch accumulation of two malting barley cultivars. Aust. J. Plant Physiol., 23: 201-210.

Schwartzkopf-Genswein, K. S., Beauchemin, K. A., McAllister, T. A., Gibb, D. J., Streeter, M. and Kennedy, A. D. 2004. Effect of feed delivery fluctuations and feeding time on rumen acidosis, growth performance, and feeding behavior of feedlot cattle. J. Anim. Sci., 82: 3357 -3365.

SeCan. 2006. Technical bulletins. [Online] Available: http://www.secan.com/ index.php?sv=&category=Varieties&title=index&crop=250 [1 Sep. 2007].

Shahidi, F. and Naczk, M. 2003. Phenolics in food and nutraceuticals. CRC Press, Boca Raton, FL.

Shewry, P. R. 1992. Barley: genetics, biochemistry, molecular biology and biotechnology. CAB International, Wallingford, UK.

Sikorska, M., Matlawska, I., Glowniak, K. and Zgorka, G. 2000. Qualitative and quantitative analysis of phenolic acids in *Asclepias syriaca L.* Acta. Pol. Pharm. 57: 69-72.

Silanikove, N. and Brosh, A. 1989. Lignocellulose degradation and subsequent metabolism of lignin fermentation products by the desert black Bedouin goat fed on wheat straw as a single-component diet. Br. J. Nutr., 62: 509-520.

Slafer, G. A., Molina-Cano, J. L., Savin, R., Araus and Romagosa, J. L. 2002. Barley science: recent advanced from molecular biology to agronomy of yield and quality. The Haworth Press, Inc., Binghamton, NY.

Statistics Canada. 2000-2008. Canada: barley supply and disposition. [Online] Available: http://www.agr.gc.ca/mad-dam/index_e.php?s1=pubs&s2=go-co&page= go-co-hist [20 March, 2008].

Statistics Canada. 2003-2005. Field crop reporting series, 22-002-X. [Online] Available: http://www.statcan.ca/cgi-bin/downpub/freepub.cgi?subject=920#920 [25 Sep. 2007].

Stewart, D., Robertson, G. W. and Morrison, I. M. 2005. Phenolic acids dimers in the cell walls of barley. Biol. Mass Spectrom., 23: 71–74.

Sun, R., Sun, X. F., Wang, S. Q., Zhu, W. and Wang, X. Y. 2002. Ester and ether linkages between hydroxycinnamic acids and lignins from wheat, rice, rye, and barley straws, maize stems, and fast-growing poplar wood. Ind. Crop. Prod., 15: 179-188.

Susmel, P. and Stefanon, B. 1993. Aspects of lignin degradation by rumen microorganisms. J. Biotechnol., 30: 141-148.

Sutton, J. D., Broster, W. H., Napper, D. J. and Siviter, J. W. 1985. Feeding frequency for lactating cows: Effects on digestion, milk production and energy utilization. Br. J. Nutr., 53: 117 -130.

Svihus, B., Ulen, A. K. and Harstad, O. M. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch. Anim. Feed Sci. Technol., 122: 303-320.

Tamminga, S., van Vuuren, A. M., van der Koelen, C. J., Ketelaar, R. S. and van der Togt, P. L. 1990. Rumen behavior of structural carbohydrates, non-structural carbohydrates and crude protein from concentrate ingredients in dairy cows. Neth. J. Agric. Sci., 38: 513–526.

Tamminga, S., Van Straalen, W. M., Subnel, A. P. J., Meijer, R. G. M., Steg, A., Wever, C. J. G. and Block, M. C. 1994. The Dutch protein evaluation system: the DVE/OEB-system. Livestock Prod. Sci., 40: 139–155.

Vailhe, M. A. B., Provan, G. J., Scobbie, L., Chesson, A., Maillot, M. P., Cornu, A. and Besle, J. M. 2000. Effect of phenolic structures on the degradability of cell walls isolated from newly extended apical internode of tall fescue (*Festuca arundinacea Schreb.*). J. Agric. Food Chem., 48: 618-623.

Van Soest, P. J. 1994. Nutritional ecology of ruminants. 2nd ed. Comstock Pub, Ithaca, NY.

Van Soest, P. J., Roberson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Vanzant, E. S., Cochran, R. C. and Titgemeyer, E. C. 1998. Standardization of in situ techniques for ruminant feedstuff evaluation. J. Anim. Sci., 76: 2717–2729.

Varga, G. A. and Kolver, E. S. 1997. Microbial and animal limitations to fiber digestion and utilization. J. Nutr. , 127 (Suppl.): 819-823.

Voltasa, J., Eeuwijk, F. A. v., A. Sombreroc, A.Lafargad, E. Igartuae and Romagosa, I. 1999. Integrating statistical and ecophysiological analyses of genotype by environment interaction for grain filling of barley I.

individual grain weight. Field Crops Research, 62: 63-74.

Wakabayashi, K., Hoson, T. and Kamisaka, S. 1997. Osmotic stress suppresses cell wall stiffening and the increase in cell wall-bound ferulic and diferulic acids in wheat coleoptiles. Plant Physiol., 113: 967-973.

Walker, A. M. 2007. FTIR microspectroscopy as a tool for evaluating the digestibility characteristics of cereal grain fed to ruminants. M.Sc. Thesis, Department of Animal and Poultry Science, Saskatoon, SK.

Wang, Y., McAllister, T. A., Xu, Z. J., Gruber, M. Y., Skadhauge, B., Jende-Strid, B. and Cheng, K. J. 1999. Effects of proanthocyanidins, dehulling and removal of pericarp on digestion of barley grain by rumen micro-organisms. J. Sci. Food Agric., 79: 929-938.

Wells, J. E., Berry, E. D. and Varel, V. H. 2005. Effects of common forage phenolic acids on *Escherichia coli* O157:H7 viability in bovine feces. Appl. Environ Microbiol., 71: 7974-7979.

Whitmore, E. T. J. 1961. Rapid method for determination of the husk content of barley and oats. J. Inst. Brew., 66: 407-408.

Whitney, S. E. C., Gothard, M. G. E., Mitchell, J. T. and Gidley, M. J. 1999. Roles of cellulose and xyloglucan in determining the mechanical properties of primary plant cell walls. Plant Physiol., 121: 657-663.

Wilson, J. R. and Hatfield, R. D. 1997. Structural and chemical changes of cell wall types during stem development: consequences for fibre degradation by rumen microflora. Aust. J. Agric. Res., 48: 165-180.

Yang, W. Z., Beauchemin, K. A., Farr, B. I. and Rode, L. M. 1997. Comparison of barley, hull-less barley, and corn in the concentrate of dairy cows. J. Dairy Sci., 80: 2885-2895.

Yang, W. Z., Beauchemin, K. A. and Rode, L. M. 2000. Effects of barley grain processing on extent of digestion and milk production of lactating cows. J. Dairy Sci., 83: 554-568.

Yu, P. 2005. Modeling nutrient supply to dairy cattle from a feedstuff using NRC-2001 (a TDN-based model) with inputs based on in situ and mobile bag technique measurements. Can. J. Anim. Sci., 85: 513-519.

Yu, P., Doiron, K. and Liu, D. 2008. Shining light on the differences in molecular structural chemical makeup and the cause of distinct degradation behavior between malting- and feed-type barley using synchrotron FTIR microspectroscopy: A novel approach. J. Agric. Food Chem., 50: 3417 - 3426

Yu, P., Maenz, D. D., McKinnon, J. J., He, T., Racze, V. J. and Christensen, D. A. 2002. Release of ferulic acid from oat hulls by *Aspergillus* ferulic acid esterase and *Trichoderma* xylanase. J. Agric. Food Chem. 50: 1625–1630.

Yu, P., McKinnon, J. J. and Christensen, D. A. 2005a. Hydroxycinnamic acids and ferulic acid esterase in relation to biodegradation of complex plant cell walls. Can. J. Anim. Sci., 85: 255 -267.

Yu, P., McKinnon, J. J. and Christensen, D. A. 2005b. Improving the nutritional value of oat hulls for ruminant animals with pretreatment of a multienzyme cocktail: In vitro studies. J. Anim. Sci., 83: 1133-1141.

Yu, P., Meier, J. A., Christensen, D. A., Rossnagel, B. G. and McKinnon, J. J. 2003. Using the NRC-2001 model and the DVE/OEB system to evaluate nutritive values of Harrington (malting-type) and Valier (feed-type) barley for ruminants. Anim. Feed Sci. Technol., 107: 45-60.

Zarra, I., Sanchez, M., Pena, M. J. and Revilla, G. 1999. The cell wall stiffening mechanism in *Pinus pinaster Aiton*: regulation by apoplastic levels of ascorbate and hydrogen peroxide. J. Sci. Food Agric., 79: 416-420.

Zhang, Y., Darlington, H., Jones, H. D., Halford, N. G., Napier, J. A., Davey, M. R., Lazzeri, P. A. and Shewry, P. R. 2003. Expression of the gamma-zein protein of maize in seeds of transgenic barley: effects on grain composition and properties. Theor. Appl. Genet., 106: 1139 -1146.

Zinn, R. A., Montano, M. and Shen, Y. 1996. Comparative feeding value of hulless vs covered barley for feedlot cattle. J. Anim. Sci., 74: 1187-1193.

Zupfer, J. M., Churchill, K. E., Rasmusson, D. C. and Fulcher, R. G. 1998. Variation in ferulic acid concentration among diverse barley cultivars measured by HPLC and microspectrophotometry. J. Agric. Food Chem., 46: 1350-1354.

Completed Projects

Part II

Project 4: PARTICLE SIZE, CHEMICAL PROFILE AND IN SITU RUMEN DEGRADATION KINETICS OF DRY MATTER, CRUDE PROTEIN AND STARCH AMONG SIX BARLEY VARIETIES: EVALUATION AND CORRELATION

Project Details

Introduction

Barley is widely used as an ingredient for animal feeding in North America. Canada contributed to world barley production with 11.8 million metric tonnes and ranked second in the world in 2007 (FAO 2007). Saskatchewan produced 4,273,000 out of a total of 10,580,000 tonnes of barley production in western Canada for the 1999-2008 annual average (Langrell et al. 2008). Various barley cultivars have been developed for different purposes. Published results about barley digestibility for ruminants show that there is a great deal of variation in the nutrient value among barley varieties. Barley varieties exhibit different rumen degradation characteristics and nutrient availabilities even though their chemical composition may be similar (Khorasani et al. 2000; Yu et al. 2003).

It has been documented that particle size negatively influences the rumen degradation rate (Nocek and Tamminga 1991). Feeds with smaller particle size have relatively greater surface area which can be accessed by rumen microbes and enzymes. This may result in a faster digestion rate. Excessive ruminal fermentation may lead to an undesired rumen pH drop. Since low rumen pH results from faster degradation of concentrates and can cause metabolic disorders, more attention should be paid to the evaluation of processing methods (Ørskov 1986). Appropriate particle size of feed is required for ruminants since it improves nutrient availability and reduces the risk of digestive disorders. This benefits both the rumen microorganisms and the host animal.

Objectives

The objectives of this study were: 1) to detect the difference of mean and median particle size of six barley varieties grown in Western Canada; 2) to determine the nutrients (DM, CP and ST) contents and degradation kinetics of six barley varieties using *in situ* technique; 3) to detect relationship between the mean and median particle size and nutrient degradability and availability.

Materials and methods

Barley Varieties and Processing Method

Six barley varieties were used in this study, including AC Metcalfe (malting purpose), CDC Dolly (feed purpose), McLeod (feed purpose), CDC Helgason (feed purpose), CDC Trey (feed purpose), CDC Cowboy (feed and forage purpose). All are two-row hulled spring barley varieties. AC Metcalfe has higher yield, larger and plumper seeds than Harrington. CDC Dolly is widely grown in Canada for its high test weight and plumpness. McLeod is developed with good yield and higher test and kernel weight than Harrington. CDC Helgason has similar plumpness, but higher yield and test weight compared to CDC Dolly. CDC Trey is suitable for growing in the eastern Canada Prairies with large and plump seeds. CDC Cowboy has high biomass but somewhat lower grain yield. (B. G. Rossnagel, personal communication 2009; Rossnagel and Harvey 1994; Legge 2003).

The year of the barley grain harvest was 2005. The climate conditions during the year were 17.5°C mean maximum daily temperatures and 455 mm rainfall. Barley samples were obtained from the Crop Development Center (CDC), University of Saskatchewan. All barley varieties were grown without irrigation at the Kernen Crop Research Farm, University of Saskatchewan, Canada, following standard agronomic production practices for barley production. The barley samples were coarsely dry rolled using a 130-teeth roller mill (Sven products, Apollo Machine and products Ltd. Saskatoon, Canada) through 1.59 mm gap with 384 rpm.

Particle Size Analysis

The particle size data for 2005 year barley samples were reported by Du (2009). Particle size analysis was performed using Pond's Model: $R = 100 \times e^{-k(s-w)}$, where, R = percentage cumulative weight oversize; s = sieve opening size (mm) (six test sieves, aperture size: 3.36, 2.36, 1.70, 1.19, 0.84, and 0.58 mm); w = the smallest predictable particle size; k = the decay constant of the exponential curve describes the proportionality constant between the percent of particles passed to the next sieve and the percent remained. More details were summarized in Du (2009).

Animal Work

Animals and Diets:

Three mature Holstein dry cows were used to measure rumen degradation characteristics. The cows were ruminally cannulated (Bar Diamond Inc., Parma, ID, USA) (internal diameter 10 cm). During the *in situ* experiment period, the cows were housed in pens of approximately 5 m× 5 m. The cows were individually fed twice per day at 0800 and 1600, with 15 kg (7.5 kg at each feeding time) of a total mixed ration consisting of 75% barley silage and 25% pelleted concentrate (as fed). Access to water was *ad libitum*. The care, maintenance and surgical techniques of the animals used in this experiment were carried out according to the guidelines of the Canadian Council on Animal Care (1993).

Rumen Incubation:

The *in situ* technique was used to determine the rumen degradability of the six barley varieties. Seven gram samples were weighed into each coded nylon bags (10 cm×17 cm, ScreenTech Corp, Mississauga ON), then incubated in the rumen for 0, 2, 4, 8, 12, 24 and 48 h according to the "gradual addition/all out" schedule (Yu et al. 2003). The pore size of nylon bag was approximately 40 μm. Sample weight to bag area ratio was approximately 41 mg/cm^2. After incubation, the bags were removed from the rumen and rinsed in cold water without detergent to remove excess ruminal contents. The 0-h incubation samples were washed with the same procedure. Subsequently, all sample bags were dried at 55°C for 48 h. Dry samples were stored at 4 °C until analysis (Yu et al. 2003).

Chemical Analysis

Dry matter was determined by drying at 105°C until the sample weight was constant according to the procedure of Association of Official Analytical Chemists (AOAC) Of[illegible] Method 930.15 (1997). Protein was analyzed by using the LECO FP-528 (LECO Corporation, 3000 Lakeview Ave. St. Joseph, MI, USA) according to the procedure of AOAC Official Method 990.03 (1997). Starch was measured with Megazyme Total Starch Assay Kit (Megazyme international Ireland Ltd., Co. Wicklow, IE) according to the procedure of AOAC Official Method 996.11 (1997).

Rumen Degradation Kinetics

The first order kinetics degradation model (Ørskov and McDonald 1979) modified by Tamminga et al. (1994) was used to describe the rumen degradation characteristics of these six barley varieties as follows:
DM and CP: $R(t) = U + (100 - S - U) \times e^{-Kd \times (t-T0)}$,
Non-structural carbohydrate -Starch: $R(t) = (100 - S) \times e^{-Kd \times t}$
Where, R(t) is residue of the incubated material after t h of rumen incubation (%), S is rapidly degradable fraction (%), D is slowly degradable fraction (%), U is undegradable fraction, Kd is degradation rate (%/h), Kp is passage rate for concentrate (6%/h), adopted by Tamminga et al. (1994), and T0 is lag time (h).

The effective degradability (ED) values were calculated as:
EDDM (or EDCP) (%) = $S + D \times Kd/(Kp + Kd)$,
EDST (%) =100 - RUST (%) = $100 - U - D \times Kp/(Kp + Kd) = S + D \times Kd/(Kd + Kp)$,
The rumen undegraded feed DM (RUDM), protein (RUP) and starch (RUST) values were calculated as:
RUDM (or RUP) (%) = $U + D \times Kp/(Kp + Kd)$,
RUST (%) = $D \times Kp/(Kp + Kd) + 0.1 \times S$ (Tamminga et al. 1994).

The NLIN procedure of the statistical package SAS software 9.1.3 (SAS Institute, Inc., Cary, NC) (SAS 2003). with iterative least squares regression (Gauss–Newton method) will be used to describe its degradation accurately. The NLIN program in this study was written by Dr. Yu, Peiqiang (University of Saskatchewan).

Statistical Analysis

Statistical analyses were performed using the MIXED procedure of SAS 9.1.3 software (SAS Institute, Inc., Cary, NC). The model used for the analysis was: $Y_{ijk} = \mu + F_i + S_j + e_{ijk}$, where, Y_{ijk} was an observation of the dependent variable ijk; μ was the population mean for the variable; F_i was the effect of barley varieties, a fixed effect; S_j was the replicate effect, a random effect; and e_{ijk} was the random error associated with the observation ijk. Fisher's protected LSD test ($P < 0.05$) was applied to compare treatment means. Proc Corr procedure of SAS package was conducted to examine the correlation between particle size data and degradation characteristics.

Results and Discussion

Comparison of Mean and Median Particle Size of Coarsely Dry-Rolled Barley Grains among Six Barley Varieties

The mean and median particle size parameters of six barley varieties are given in Table . AC Metcalfe and CDC Trey had the smallest ($P<0.05$) mean (2.82 mm and 2.93 mm, respectively) and median (2.45 mm and 2.55 mm, respectively) particle size among the six varieties. In contrast, CDC Helgason and CDC Dolly had larger ($P<0.05$) particle sizes (means: 3.09 mm and 3.09 mm; medians: 2.75 mm and 2.74 mm, respectively). Although CDC Trey is a barley variety for feed purpose, it was similar in particle size to AC Metcalfe, a barley variety for malting purpose.

Table 1. Particle size comparison of six barley varieties

Items	Barley variety[z]							
	AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy	SEM[y]	P value
Particle sizes								
Mean (mm)	2.82 c	3.09 a	3.06 ab	3.09 a	2.93 bc	3.05 ab	0.044	0.0056
Median (mm)	2.45 c	2.74 a	2.66 ab	2.75 a	2.55 bc	2.61 ab	0.048	0.0057

[z] All six varieties of barley were grown at the Kernen Crop Research Farm (University of Saskatchewan, Saskatoon, Canada) and were managed using the same and standard agronomic production practices for all barley production (Crop Development Center).

[y] SEM= standard error of mean. Means with the different letter in the same row are significantly different ($P<0.05$). Mean separation was done by using the Fisher's protected LSD test method.

Cereal processing can modify particle size of a feedstuff, which may influence particle passage rate and degradation rate and consequently, the entire digestion process (Nocek and Tamminga 1991). Larger particle size enhances palatability (Theurer 1986; Church 1991; Kellems and Church 2002). Additionally, the larger particle size may somewhat reduce problems arising from the fast ruminal degradation (Ørskov 1986). As previously reported, differences in particle size was related to the ADF content in grain (Bowman et al. 2001). More research is needed to address the problem of how the chemical and structural composition affect the particle size parameters, and subsequently to understand how chemical conformation influence nutrient digestibility.

Evaluation of Chemical Profile and *In Situ* Rumen Degradation Kinetics of Dry Matter, Crude Protein and Starch among Six Barley Varieties

The degradation kinetics of six barley varieties were determined using the modified Ørskov model (Tamminga et al. 1990; Tamminga et al. 1994). The results are presented in Table 2.. DM content (%) was similar among the six barley varieties. There were significant differences in DM degradation features among these six barley varieties. The results showed that the barley varieties differed in all degradation characteristics (S, D, U, RUDM and EDDM) except for T0. The S differed ($P<0.01$) from 0.2% (CDC Helgason) to 1.2% (CDC Trey), indicating that CDC Trey, a barley variety for feed purpose, contains higher soluble nutrients. Additionally, the D fraction differed in that CDC Dolly had the lowest ($P<0.05$) D fraction (69.1%) while AC Metcalfe, CDC Trey and CDC Cowboy had the highest D fractions (77.0, 79.0 and 77.6%, respectively). It also showed that these six varieties had similar ($P>0.05$) T0. There were marked differences ($P<0.01$) in Kd and extent (EDDM) ($P<0.01$) among the barley varieties. In comparison with other barley varieties, the Kd of CDC Helgason was lowest (5.62 %/h) ($P<0.01$), followed by CDC Dolly, of which the Kd was 7.84 %/h. The remaining four barley varieties showed relatively faster Kd (10.04 %/h for AC Metcalfe, 9.72 %/h for CDC Trey, 9.63 %/h for McLeod and 8.29 %/h for CDC Cowboy).

Table 2. Comparison of six barley varieties: chemical profile and *in situ* rumen degradation kinetics of dry matter (DM).

	Barley variety[z]							
Items	AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy	SEM[y]	P value
In situ rumen degradation kinetics of DM[x]								
Dry matter (DM, %)	91.89	91.78	91.76	91.96	91.94	91.82	-	-
S (%)	0.81 ab	0.39 cd	0.62 bc	0.20 d	1.16 a	0.63 bc	0.146	0.0065
D (%)	76.99 ab	69.05 d	72.10 cd	74.44 bc	79.03 a	77.55 ab	1.512	0.0049
U (%)	22.20 cd	30.56 a	27.28 ab	25.36 bc	19.80 d	21.81 cd	1.576	0.0040
T0 (h)	0.91	1.34	1.00	0.90	0.95	1.04	0.199	0.6555
Kd (%/h)	10.04 a	7.84 b	9.63 ab	5.62 c	9.72 a	8.29 ab	0.671	0.0025
RUDM (g/kg DM)	513.4 d	605.1 b	550.0 c	640.2 a	502.0 d	545.4 c	9.99	<0.0001
EDDM (g/kg DM)	486.7 a	394.9 c	450.0 b	359.8 d	498.0 a	454.6 b	9.99	<0.0001

[z] All six varieties of barley were grown at the Kernen Crop Research Farm (University of Saskatchewan, Saskatoon, Canada) and were managed using the same and standard agronomic production practices for all barley production (Crop Development Center).

[y] SEM= standard error of mean. Means with the different letter in the same row are significantly different ($P<0.05$). Mean separation was done by using the Fisher's protected LSD test method.

[x] S= rapidly degradable fraction; D= potentially degradable fraction; U= undegradable fraction; Kd = degradation rate; RUDM= rumen undegradable DM; EDDM= effective degradability of DM.

CDC Trey and AC Metcalfe were the highest ($P<0.01$) in EDDM (498 and 487 g/kg base on DM, respectively). In contrast, CDC Helgason was the lowest in EDDM (360 g/kg base on DM). CDC Helgason had the largest RUDM (640 g/kg DM), whereas the CDC Trey and AC Metcalfe exhibited the smallest RUDM (502 and 513 g/kg base on DM, respectively). These results indicated that the six barley varieties had different ruminal degradation characteristics. Earlier research indicated that the ruminal DM digestibility of barley grain that has been dry rolled varies between 82-621 g/kg (Bowman et al. 2001). A slower degradation rate could result in more bypass nutrients to the host animal and reduce the risk of digestive disorders (Khorasani et al. 2000). Therefore, CDC Helgason, with the slowest degradation rate and smallest EDDM, may supply relatively more nutrients into the small intestine of ruminants. In contrast, other varieties may have more DM digested in the rumen prior to entering the small intestine. In particular, the degradation behaviour of CDC Trey exhibited similar degradation kinetics (S, D, U, Kd, RUDM, EDDM) of DM with AC Metcalfe, implying that it may not be ideal for feed purpose. Additionally, AC Metcalfe and CDC Trey, the two varieties with the smallest mean and median particle size, had the highest Kd and EDDM, suggesting a relationship between degradation kinetics and particle size.

The *in situ* CP degradation results are given in Table . Protein content varied in the range of 12.4-14.4%, which is consistent with earlier findings (Khorasani et al. 2000; Holtekjøen et al. 2006). AC Metcalfe had the greatest ($P<0.05$) S protein fraction (3.7%). The other five varieties ranged from 0.7 to 2.4%. CDC Dolly and McLeod had relatively smaller ($P<0.05$) D fraction (73.4 and 78.1%, respectively). CDC Dolly and McLeod had a relatively greater ($P<0.05$) U fraction (24.7 and 21.7%, respectively). T0 for AC Metcalfe and CDC Dolly were 0.84 and 1.76 h, respectively. Others were intermediate, ranging from 1.27 to 1.49 h. Slower ($P=0.056$) Kd of CP was found on CDC Helgason (4.55 %/h). The degradation rate of CDC Trey was 6.95 %/h, which was numerically the highest among the six barley varieties.

Table 3. Chemical profile and *in situ* rumen degradation kinetics of crude protein (CP): Comparison of six barley varieties.

	Barley variety[z]							
Items	AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy	SEM[y]	P value
In situ rumen degradation kinetics of CP[x]								
Crude protein (CP, %DM)	14.11	13.08	13.36	13.19	12.35	14.39	-	-
S (%CP)	3.73 a	1.97 b	0.76 c	2.11 b	0.71 c	2.40 b	0.360	0.0002
D (%CP)	78.38 abc	73.35 c	78.05 bc	79.89 ab	83.76 a	81.45 ab	2.194	0.0270
U (%CP)	17.89 b	24.68 a	21.19 ab	18.00 b	15.54 b	16.14 b	2.102	0.0400
T0 (h)	0.84 b	1.76 a	1.27 ab	1.47a b	1.49a b	1.40a b	0.283	0.3705
Kd (%/h)	6.72 a	6.18 ab	6.99 a	4.55 b	6.95 a	5.73 ab	0.551	0.0560
RUP (%CP)	55.31 c	60.96 ab	57.44 bc	63.59 a	54.70 c	57.83 bc	1.250	0.0022
RUP (g/kg DM, DVE[w])	86.6 bc	88.5 abc	85.2 c	93.1 a	75.0 d	92.4 ab	1.88	0.0002
RUP (g/kg DM, NRC[v])	78.0 bc	79.7 abc	76.7 c	83.9 a	67.5 d	83.2 ab	1.69	0.0002
EDCP (%CP)	44.69 a	39.04 bc	42.56 ab	36.41 c	45.30 a	42.17 ab	1.250	0.0022
EDCP (g/kg DM)	63.1 a	51.1 cd	56.9 b	48.0 d	55.9 bc	60.7 ab	1.69	0.0003

[z] All six varieties of barley were grown at the Kernen Crop Research Farm (University of Saskatchewan, Saskatoon, Canada) and were managed using the same and standard agronomic production practices for all barley production (Crop Development Center).

[y] SEM= standard error of mean. Means with the different letter in the same row are significantly different ($P<0.05$). Mean separation was done by using the Fisher's protected LSD test method.

[x] S= rapidly degradable fraction; D= potentially degradable fraction; U= undegradable fraction; Kd = degradation rate; RUP = rumen undegradable CP; EDCP = effective degradability of CP.

[w] RUP was estimated using the DVE/OEB system (Tamminga et al. 1994).

[v] RUP was estimated using the NRC 2001 model (NRC 2001).

In comparison with the other four varieties, CDC Helgason (63.6%) and CDC Dolly (61.0%) had a higher ($P<0.05$) percentage of rumen undegradable CP (RUP %). Compared with the other barley varieties, CDC Trey showed the lowest ($P<0.01$) RUP (g/kg DM) calculated from the two models (75 g/kg DM from DVE/OEB model and 68 g/kg DM from NRC 2001 model, respectively). The results showed that both the NRC 2001 model and the DVE/OEB model could be used to detect the difference of RUP among the six barley varieties. CDC Helgason and CDC Dolly had lower ($P<0.01$) percentage of EDCP (36.4 and 39.0%, respectively). Others ranged from 42.2 to 45.3%. The EDCP (g/kg DM) of these two varieties were lower than others as well (48 and 51 g/kg DM, respectively), while AC Metcalfe and CDC Cowboy were higher (63 and 61 g/kg DM, respectively). Comparing with other earlier study, the results appeared higher in EDCP than that of steam-rolled barley (Yang et al. 1997). It is conclusive that protein digestibility of different barley varieties differed significantly in the rumen. CDC Helgason may be less extensively fermented in the rumen compared to the other five barley varieties.

The *in situ* starch results (Table 4) show that the starch percentage of CDC Dolly (58.6%) compared closely with a previous study of Holtekjøen et al. (2006), but CDC Helgason was lower (56.4%). More variable results of barley varieties were reported earlier (Herrera-Saldana et al. 1990; Khorasani et al. 2000). Previous work indicated that type and amount of polysaccharides impact starch digestibility (Nocek and Tamminga 1991). In this study, the Dutch model (Tamminga et al. 1994) was used to assess the degradation of starch. The results indicate that the starch rate and extent of degradation of six barley varieties were significantly different, though they had similar D fractions ($P>0.05$). CDC Helgason had the lowest ($P<0.05$) Kd (3.67%/h), which indicates that the starch release of CDC Helgason was markedly slower than that of the other five barley varieties in the rumen. In contrast, CDC Trey had the highest Kd of starch (7.11%/h). The degradation rate of the remaining four varied from 4.24 to 7.05%/h, lower than the previous data obtained from steam-rolled barley (Foley et al. 2006) likely because of different processing methods. It has been interpreted that barley starch can be degraded extensively in the rumen (Waldo 1973). However in the small intestine, starch may be utilized to generate more energy (Bowman et al. 2001) by reducing methane production. There were significant differences observed for the percentage of RUST as well. CDC Helgason revealed the highest ($P<0.01$) percentage of RUST (61.0%). In addition, for RUST (g/kg DM), CDC Helgason and CDC Dolly were relatively higher (344 and 342 g/kg DM, respectively). Differences between varieties were particularly apparent when their EDST were compared. AC Metcalfe and CDC Trey showed higher ($P<0.05$) EDST percentage (55.0 and 54.3%, respectively) compared with other barley varieties, whereas CDC Helgason was the smallest (39.0%). AC Metcalfe showed the highest ($P<0.05$) EDST (322 g/kg DM), comparing with the relatively smaller CDC Helgason (220 g/kg, DM) and McLeod (228 g/kg DM). The others are intermediate. This is also evidence that their ruminal degradation characteristics varied between varieties.

This use of the *in situ* technique to determine the ruminal degradability of barley grain indicated that these six barley varieties exhibited different digestion kinetics characteristics, even though they were grown and processed under the same conditions. This is consistent with the previously published results (Hart et al. 2008; Du et al. 2009). Although the barley digestibility for ruminants has been studied extensively, the ruminal degradation kinetics parameters reported in this study are not very comparable with other reports due to the variation of sample pre-treatment, laboratory conditions and analysis methodologies (Yang et al. 1997; Khorasani et al. 2000; Bowman et al. 2001; Yu et al. 2003; Hindle et al. 2005; Foley et al. 2006; Miller et al. 2007; Du et al. 2009).

Compared with the other five barley varieties, the degradation characteristics of CDC Helgason, in terms of the degradation rate and extent, are extremely attractive. CDC Helgason had the largest rumen undegradable fraction of DM (640 g/kg DM), CP (84 g/kg DM, NRC) and starch (344 g/kg DM) and lowest degradation rate of DM (5.62%/h), CP (4.55%/h) and starch (3.67%/h), respectively. The relatively lower degradation rate and extent may reduce issues with rumen acidosis. These comparative advantages support a perspective that CDC Helgason may be ideal for feed purpose compared with the other five barley varieties.

Table 4. Chemical profile and *in situ* rumen degradation kinetics of starch (ST): Comparison of six barley varieties.

	Barley variety[z]							
Items	AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy	SEM[y]	P value
In situ rumen degradation kinetics of ST[x]								
Starch (ST, %DM)	58.44	58.59	48.81	56.39	55.67	54.15	-	-
S (%ST)	2.93	0.51	0.01	2.14	0.18	0.09	1.117	0.2509
D (%ST)	97.07	99.49	99.99	97.86	99.82	99.91	1.117	0.2509
Kd (%/h)	7.05 a	4.24 d	5.28 c	3.67 e	7.11 a	6.16 b	0.176	<0.0001
RUST (%ST)	44.96 e	58.33 b	53.21 c	61.01 a	45.73 e	49.31 d	0.870	<0.0001
RUST (g/kg DM)	262.7 b	341.7 a	259.7 b	344.0 a	254.6 b	267.0 b	4.91	<0.0001
EDST (%ST)	55.04 a	41.67 d	46.79 c	38.99 e	54.27 a	50.69 b	0.870	<0.0001
EDST (g/kg DM)	321.7 a	244.2 d	228.4 e	219.9 e	302.1 b	274.4 c	4.91	<0.0001

[z] All six varieties of barley were grown at the Kernen Crop Research Farm (University of Saskatchewan, Saskatoon, Canada) and were managed using the same and standard agronomic production practices for all barley production (Crop Development Center).

[y] SEM= standard error of mean. Means with the different letter in the same row are significantly different ($P<0.05$). Mean separation was done by using the Fisher's protected LSD test method.

[x] S= rapidly degradable fraction; D= potentially degradable fraction; U= undegradable fraction; Kd = degradation rate; RUST = rumen undegradable starch; EDST= effective degradability of starch.

An earlier study reported that few differences in chemical composition and starch degradation kinetics were found between AC Metcalfe and the mean of five feed-type barley varieties (Hart et al. 2008). However, with respect to degradation features obtained in this study, undoubtedly, AC Metcalfe, with greater soluble fraction, faster degradation rate and greater effectively degradability, showed its suitability for use in malting. The protein matrix in barley grain was assumed to play a protective role against degradation (Holopainen et al. 2005). Although AC Metcalfe appeared to have numerically higher protein content (14.1%), it is still the most ruminally degradable among the six varieties. Associated with its small particle size, different structural and chemical structure may explain this. The protein matrix of AC Metcalfe may be highly soluble and more easily dissolved in rumen fluid, which can lead to greater ruminal digestibility. In addition, the loose protein-starch conjunction within cellular dimension might imply a faster degradation rate, and result in increased nutrient availability in rumen.

AC Metcalfe, a barley variety for malting purpose, exhibited faster degradation rates of DM (10.04%/h) ($P<0.01$), CP (6.72%/h) ($P=0.056$) and starch (7.05%/h) ($P<0.01$) among the six barley varieties. However, CDC Trey, a barley variety for feed purpose, also had similar degradation features (9.72 for DM, 6.95 for CP, and 7.11%/h for starch, respectively) to AC Metcalfe. Based on these observations, CDC Trey was degraded in the rumen more like a barley variety for malting purpose. Moreover, a large portion (54.3, vs. 55.0% of AC Metcalfe) of starch of CDC Trey was effectively digested in the rumen, while only 45.7% (vs. 45.0% of AC Metcalfe) escaped from ruminal microorganism fermentation. Excessively rapid digestion of starch in the rumen may cause metabolic disorders. It can be argued that CDC Trey may not be suitable for feeding.

Correlation between Mean and Median Particle Sizes and Degradation Rate and Extent of Dry Matter, Crude Protein and Starch among Barley Varieties

The rumen degradation rate and extent are of particular interest to evaluate the quality of grains. The particle size of processed barley grain may be partly responsible for the degradation behaviour (Kellems and Church 2002). In order to further address this problem, Pearson correlation was determined between particle size parameters and degradation rate and extent (Table 1). Negative correlations were observed between the median particle size and effective digestibility of DM ($P<0.05$) and CP ($P<0.05$). Compared with the mean particle size, median particle size revealed relatively strong ($P<0.01$) negative correlations with degradation rate and extent of starch degradation. The median particle size can therefore be considered to be correlated with the rumen fermentation of barley grain. Kd of DM tended to be correlated with median particle size but Kd of CP did not. No significant correlations were detected between mean particle size and starch degradation features (Kd and EDST).

This leads to the conclusion that median particle size, not mean particle size, is associated with the degradation rate and extent of processed barley. These results agree with the earlier conclusion that the large particles are related to slower ruminal degradation (Nocek 1988). Additionally, the results strengthen the concept that the particle size of barley can affect digestibility. Processing destroys the protein starch matrix (Figure 1) which surrounds the starch granules and prevents the access of microbial enzymes. Taking the ruminal microbial community into account, smaller particle size implies larger surface area, to which microorganisms and digestive enzymes can efficiently attach. Additionally, smaller particle size may also contribute to the high passage rate. Whereas larger particles appear to need more time to be further broken down in the digestion process with a smaller accessible surface area (Theurer 1986; Nocek and Tamminga 1991; Bowman et al. 2001). There was evidence that particle size has an effect on ruminal passage rate of forage (Welch 1986; Sniffen et al. 1992). Based on related studies (Yoshimoto et al. 2000; You and Izydorczyk 2002; Tester et al. 2004), it is possible that large particle size results from the tight protein structure combining with the starch granules. A speculation was also made that CDC Helgason with larger particle size may be relatively resistant against ruminal degradation and still fermentable in lower gut. As a result, it is probably the most suitable barley variety for feed purpose for ruminants among these six varieties.

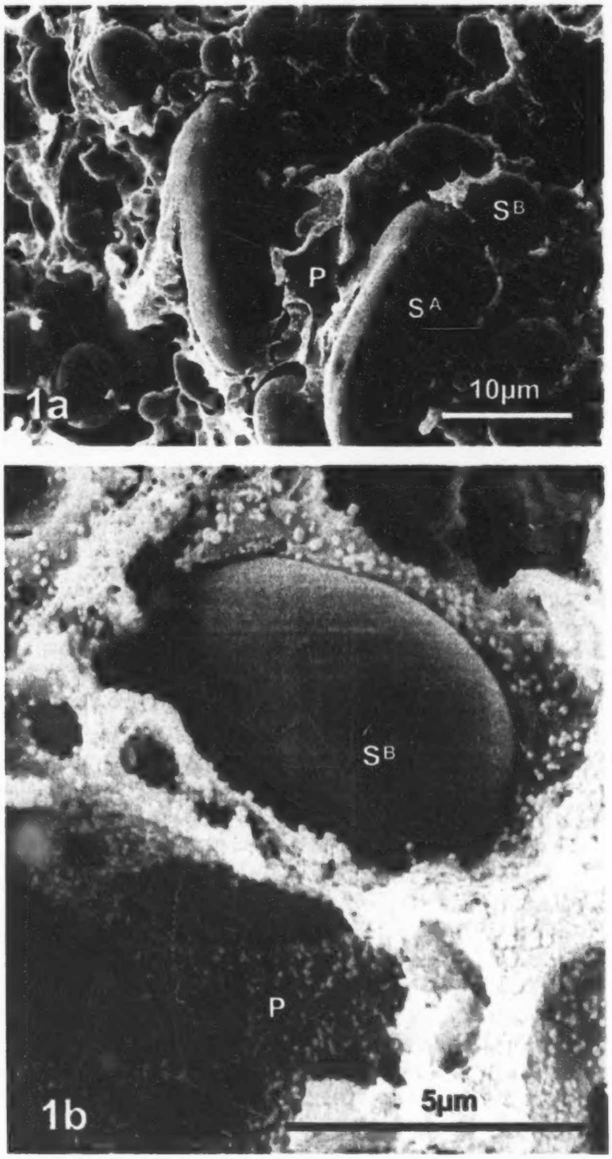


Figure 1. Scanning electron micrograph of mature starchy endosperm cells of wheat. (a) Tissue from the central starchy endosperm of cv Bouquet showing small (SB) and large (SA) starch granules embedded in a protein matrix (P). (b) Enlargement to show the protein matrix (P) in starchy endosperm tissue of cv Avalon. (Source: Mills et al. 2005)

Table 1. Pearson correlations between *in situ* rumen degradation rate and extent of dry matter (DM), crude protein (CP) and starch (ST) from six barley varieties and mean and median of particle sizes.

	Mean particle size[z]		Median particle size[z]	
Items	Correlation coefficient	P value	Correlation coefficient	P value
In situ rumen degradation kinetics of DM[y]				
Kd (%/h)	-0.774	0.071	-0.784	0.065
EDDM (%)	-0.760	0.079	-0.894	0.016
In situ rumen degradation kinetics of CP[y]				
Kd (%/h)	-0.799	0.057	-0.593	0.215
EDCP (%)	-0.784	0.065	-0.885	0.019
In situ rumen degradation kinetics of ST[y]				
Kd (%/h)	-0.686	0.133	-0.955	0.003
EDST (%)	-0.658	0.156	-0.954	0.003

[z] Mean and median particle sizes were determined by Pond's model.

[y] Kd = degradation rate; EDDM, EDCP, EDST= effective degradability of DM, CP and starch, respectively.

Regression analysis between median particle size and *in situ* degradation features of DM, CP, and starch was conducted using Proc Mixed procedure of SAS package to give a possible answer regarding the magnitude of particle size affecting the various digestion behaviours. The results are presented in Table 6. The results showed that the four regression models are significant ($P<0.05$). It was indicative that 79.9% effective digestibility of DM, 78.3% of CP and 91.0% of ST could be explained by median particle size, respectively.

Table 2. Regression analysis to predict rumen degradability using median particle size (x) with regression model as follows: Model: Y (degradability) = Median.

Predicted variables (Y)	Model: Y= a + b*x	R^2 value	RSD [z]	P value
EDDM	Y = 153.00 – 41.47 * x	0.799	2.68	0.016
EDCP	Y = 110.28 – 26.11 * x	0.783	1.77	0.019
Kd (ST)	Y = 51.04 – 15.96 * x	0.912	0.64	0.003
EDST	Y = 195.48 - 54.68 * x	0.910	2.21	0.003

[z]RSD= Residue standard deviation.

Conclusions

The results indicated that under the same growth and processing conditions (dry-rolled though 1.59 mm gap), the six barley varieties appeared to significantly differ in their digestion characteristics and mean and median particle sizes, and thus, had different nutrient supply to ruminants.

CDC Helgason is distinguished from other varieties due to its lowest degradation rate and extent of DM, CP and ST (5.62%/h, 360 g/kg for DM, 4.55%/h, 48 g/kg DM for CP, and 3.67%/h, 220 g/kg DM for starch, respectively) among the six barley varieties. Results indicate that CDC Helgason may be the desirable barley variety for feed purpose compared with the other five barley varieties.

Significant correlation was found between median particle size (not mean particle size) and the rate and extent of rumen degradation (P value was 0.016 for EDDM, 0.019 for EDCP, 0.003 for Kd of starch and 0.003 for EDST). Results indicate that the median particle size, to a greater extent than mean particle size, can affect the degradation rate and extent of barley grain. The differences in particle size of the processed barley grains may explain the various degradation characteristics exhibited in the rumen among the six barley varieties. Associated with the nutrient availability, particle size could be an important factor to affect barley feed quality, fermentation, and nutrient availability for ruminants. Therefore, preventing inappropriate processing is of importance for the ruminant feeding industry.

With similar chemical composition, the barley varieties exhibited different degradation behaviours. More information about the biological constitution of grain must be taken into account to explain this. It is not sufficient to solve this problem with only *in situ* degradation characteristics and mean and median particle sizes. Further study is required to investigate the inherent structural and chemical make-up characteristics between the barley varieties which may provide more information as to why barley varieties exhibit different biodegradation behaviours in the rumen and lower gut.

Acknowledgements

The authors would like to thank Saskatchewan Agriculture Development Fund (ADF, Project # 20040495) for financing this project and Saskatchewan Agriculture Development Fund (ADF) for Ministry of Agriculture Strategic Feed Research Chair Program.

Part II

Project 5: USING DIFFUSE REFLECTANCE FOURIER TRANSFORM INFARED SPECTROSCOPY (DRIFT) AND SYNCHROTRON-BASED FTIR MICROSPECTROSCOPY (SFTIRM) TO CHARACTERIZE SEED INHERENT STRUCTURE AMONG SIX BARLEY VARIETIES

Project Details

Introduction

The results of chapter 2 show that barley varieties exhibit different ruminal degradation rates. Knowledge of their chemical and structural differences may lead to an understanding of the reasons for these differences. Further study of the relationship of biological characteristics of barley grain in relation to spectral characteristics may provide useful understanding of how to best utilize the various varieties.

Grinding is usually required in chemical analysis. In addition, harsh chemical reagents can unavoidably destroy or even obliterate the histological structure of the sample (Wetzel and LeVine 2000, 2001). Structural and chemical information detection is beyond the capacity of traditional chemical analysis, owing to the destructive pre-treatment. These disadvantages necessitate a new and rapid tool to overcome these limitations.

As a potential analytical tool, FTIR technique is widely applied in various scientific fields. Different vibration and complex absorption modes result in different characteristic spectral features. Based on the monitored spectral signal, chemical and structural features can be revealed (Stuart 2004). FTIR technique has been applied to monitor the metabolic changes caused by D_2O in rat cerebellum tissue (Wetzel et al. 1998c). The detailed spectroscopic characterization of biological specimen has been summarized and reviewed (Jackson and Mantsch 2000; Wetzel 2000).

Conventional FTIR techniques show comparable performance to synchrotron when working with large aperture size (>20-30 μm) (Diem et al. 2004). However, it is inferior to synchrotron at ultra-spatial resolution when detecting biological or biomedical specimens (5-30 μm) (Miller et al. 2000). To overcome the weakness of conventional (thermal) light source, synchrotron light is used because it is extremely advantageous for small aperture setting (Miller and Dumas 2006).

The induction of synchrotron radiation allows simultaneous sample viewing and IR data collection at ultra-spatial resolution. Using synchrotron source to substitute for conventional thermal (globar) source, results in the potential to improve S/N ratio (Miller 2002; Miller and Dumas 2006). The combining of microscopy with FTIR spectroscopy enables observations both visually and spectroscopically. Ultra-spatial resolved synchrotron IR microspectroscopy is used to monitor the intrinsic distribution of chemical compounds in biological and biomedical specimens. Accordingly, taking advantage of the brilliance, broadband and concentration (Miller and Dumas 2006), the application of synchrotron light associated with FTIR technique makes it possible to probe further on the inherent structure of biological materials at a cellular or sub-cellular dimension without thermal noise (Dumas and Miller 2003).

Working with the SFTIR spectroscopy, high quality mapping of sample sections can be generated. For instance, chemical functional groups associated with nutrients of plant tissue from wheat, barley, corn, canola, flaxseed have been detected using FTIR microspectroscopy in conjunction of advanced synchrotron light source (Yu 2005b; 2006a; Yu et al. 2004b; 2005; 2007; 2009a). The photomicrograph and chemical compounds (protein, lipid, lignin, structural and non-structural carbohydrates) mapping of Valier (feed type) and Harrington (malting-type) barley have been obtained using SFTIR technique (Yu 2004; Yu et al. 2004a; 2004d; 2008). The protein molecular structure of alfalfa affected by Lc-gene transformation were firstly reported using SFTIR (Yu et al. 2009b).

A diversity of biological materials have been examined using conventional (globar) or advanced (synchrotron) FTIR techniques in conjunction with powerful statistical analysis methodologies. Agglomerative hierarchical cluster analysis (AHCA) and principal components analysis (PCA) can be used to treat the tremendous amount of information relating to matrix conformation, chemical and structural make-up and compound distribution. The AHCA can classify the objects into different clusters (groups) based on their similarity (Romesburg 1984; Kaufman and Rousseeuw 1990; Gan et al.2007). PCA can visibly present the analysis in scatter plots of principle components (PCs), of which maximal information of original data can be carried out in organized manner (Dunteman 1989; Jolliffe 2002; Yu 2006b). Previously for feed science application, AHCA and PCA analysis have been applied on discrimination of Harrington and Valier barley with their amide I FSD spectra (Yu et al. 2007). These two analysis methods have also been used to detect the differences between various tissues from wheat (Yu et al. 2007). Previous work indicated that these two multivariate spectral analysis methodologies could successfully distinguish flaxseed structure change caused by autoclaving (Doiron 2009).

Objectives

These mid-IR techniques enable us to establish a correlation of spectral information to structural and chemical features of plant tissue, furthermore, to feedstuff quality. The objectives of this study were: 1) using DRIFT spectroscopy to identify the differences in functional group characteristics associated with nutrient utilization in barley hull sample and whole seed sample among the six barley varieties; 2) using synchrotron-based FTIR microspectroscopy to determine structural make-up features and identify the structural differences in chemical functional groups in endosperm tissue among the six barley varieties; 3) using multivariate spectral analyses (AHCA and PCA) to detect spectral differences among the six barley varieties; 4) to analyze correlation between *in situ* nutrients degradation (rate and extent) and chemical functional groups features determined by DRIFT and SFTIRM spectroscopy.

Materials and methods

Barley Varieties and Growth Condition

The six barley varieties were the same mentioned previously in Chapter 2 (AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey, and CDC Cowboy).

Sample Preparation for DRIFT Spectroscopy

The hull samples of six barley varieties were produced using Laboratory Dehuller (Model LH 5095, Codema Inc. Minneapdis, Minnesota) at 100 psi for 30 sec, and then screened through a 1.2 mm pore size sieve to remove fines and dusts. Hull and whole seed samples from the six barley varieties were ground through 0.25 mm screen twice with Retsch Grinder ZM100 (Brinkmann Instruments Ltd, ON, Canada) and then mixed into KBr powder with a ratio of 4: 1.

DRIFT Spectroscopy

Mid-IR spectra from DRIFT spectroscopy technique were collected using Bio-Rad FTS-40 (Bio-Rad, Cambridge, MA) with a globar IR source in the Saskatchewan Structural Sciences Centre (SSSC), University of Saskatchewan.

DRIFT Data Collection and Processing

Win-IR software, the coupled computer system with Bio-Rad FTS-40 was used to record IR spectra. Spectra within the mid-IR region (ca. 4000-400 cm^{-1}) were collected with 256 coadded scans. Spectral resolution was set as 4 cm^{-1}. Background spectra were collected with the same measurement setting prior to formal spectra collection. Nicolet OMNIC software 7.3 (Spectra Tech, Madison, WI) was used to analyze spectral data. For normalization of the spectral information, baseline correction was applied on all spectra data. Outlier spectra, which might be due to the instrumental and environmental contributions, were eliminated prior to evaluation.

Sample Preparation for SFTIR Microspectroscopy

Five seeds of each barley variety (six varieties) were randomly selected to cut transversely across the endosperm tissue. The thin cross sections of tissues (ca. 6 μm) were unstained and immediately mounted on barium fluoride (BaF_2) discs (2 mm thick, 13 mm diameter, Spectral Systems, Hopewell Junction, NY, USA) for synchrotron FTIR microspectroscopic work in transmission mode.

Synchrotron-based SFTIR Microspectroscopy

The experiment was performed using a Thermo Nicolet Magna 860 Step-Scan FTIR (Thermo Fisher Scientific Inc., Waltham, MA) spectrometer equipped with a Spectra Tech Continuum IR Microscope (Spectra-Tech, Inc., Shelton, CT) and liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. The IR microspectroscopy instrument was coupled with synchrotron radiation from U2B beamline, Brookhaven National Laboratory National Synchrotron Light Source, U.S. Department of Energy (NSLS-BNL, Upton, NY).

SFTIRM Data Collection and Processing

The spectra were collected from five seeds of each barley variety. Spot samples (30-60) were randomly selected in endosperm area between 100-600 μm from outside of the seed section on each window. The spectra were collected through an aperture of 10 × 10 μm in a transmission mode within mid-IR spectral range of ca. 4000-800 cm^{-1}. The spatial resolution was set as 4 cm^{-1} and 128 scans were coadded on each spot to produce an IR spectrum. Background spectra were collected using the same measurement setting. Atlμs software (Thermo Nicolet, Madison, WI, USA) was used to produce the visual image of the sample. Nicolet OMNIC software 7.3 (Spectra Tech, Madison, WI, USA) was used to collect and analyze spectral data. After baseline correction, the absorption peak parameters (baseline, region, relative height and area) were recorded for further univariate analysis. More details on SFTIRM data collection were documented in Marinkovic et al. (2002) and Yu et al. (2004a).

Multivariate Spectral Analysis

Agglomerative hierarchical cluster analysis (AHCA) and principal component analysis (PCA) were applied using Statistica 8 (StatSoft Inc, Tulsa, OK, USA) to accomplish the multivariate spectral analyses. The Ward's algorithm method (Miller et al. 2000) and Euclidean Distance were applied to conduct the AHCA analysis and graphically present the similarity among barley varieties. The 1st principal component (PC1) vs. the 2nd principal component (PC2) scatter plots were generated to present the results.

Statistical Analysis

Statistical analyses were performed using the MIXED procedure of SAS 9.1.3 (SAS Institute, Inc., Cary, NC). Fisher's protected LSD test was used to compare means with $P < 0.05$ considered significant.

The DRIFT spectroscopic data were analyzed with a CRD model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} was an observation of the dependent variable ij; μ was the population mean for the variable; T_i was the effect of the barley varieties, as a fixed effect, and e_{ij} was the random error associated with the observation ij.

The SFTIR spectroscopic data were analyzed using a completed nested design. The model used for the analysis was: $Y_{ij} = \mu + T_i + S(T)_j + e_{ij}$, where, Y_{ij} was an observation of the dependent variable ij; μ was the population mean for the variable; T_i was the effect of the barley varieties, as a fixed effect, $S(T)_j$ is the seeds nested within treatments, as a random effect, and e_{ij} was the random error associated with the observation ij. The detailed methodology was reported in Yu (2004).

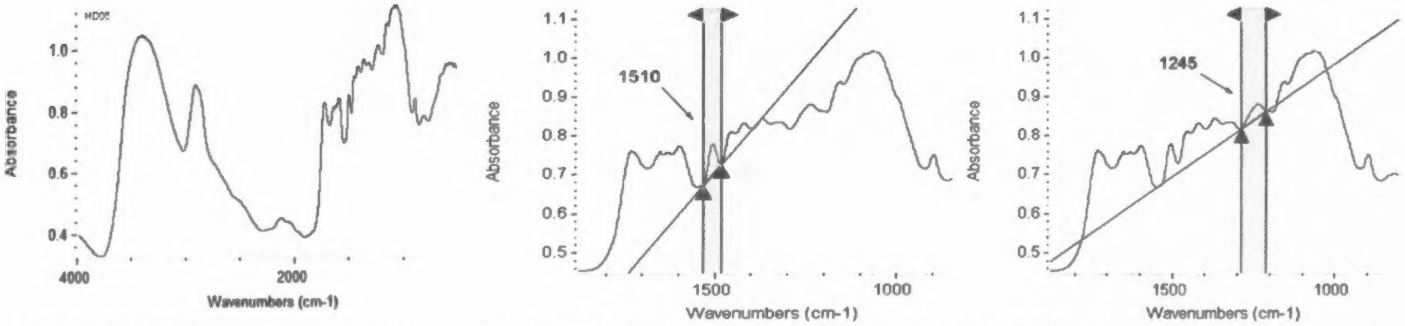
Correlation between *in situ* degradation rate and extent of DM, CP, and starch and mid-IR spectral characteristics among the barley varieties were analyzed using the Corr procedure of SAS software (SAS Institute, Inc. 2003).

Results and Discussion

The DRIFT and SFTIRM techniques were used to characterize inherent seed structure among these six barley varieties. The spectra observed in the region of 4000-800 cm^{-1} were analyzed to show the absorption characteristics attributed to the chemical functional groups of barley grains.

Using DRIFT Spectroscopy to Characterize and Compare Structure and Chemical Make-Up of Hull Samples from Six Barley Varieties

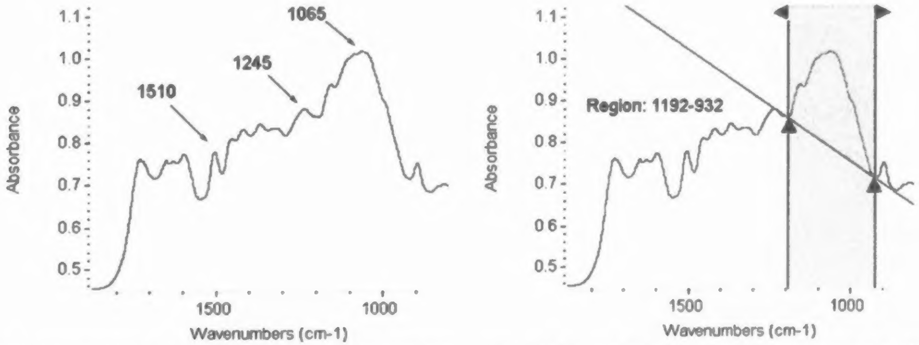
Differences in structural and chemical make-up of barley hull from six barley varieties were identified using DRIFT spectroscopy. Figure 1 exhibits a typical spectrum of hull samples from barley seeds. The complex spectrum is composed of a series of unique absorption peaks and the spectral data can be associated with the chemical compounds in barley hulls. Since certain compounds only absorb at the frequencies that match the molecular vibrations, it is possible to identify and characterize the structural and chemical make-up by spectra analysis. The detailed absorption band assignments (baseline and boundaries definitions) are shown in Figure 1, including defined boundaries of peak areas. The region 1800-800 cm^{-1} (Figure 1 b) can be considered as fingerprint region because almost all molecules present characteristic peaks in this region. Lignin exhibits obvious IR absorption band (Figure 1 c) which is centered at ca. 1510 cm^{-1} (Wetzel et al. 1998a; Dumas et al. 2004). As shown in Figure 1 (d), there is a strong and complex peak in the range of 1192-932 cm^{-1}, attributed to carbohydrates (Wetzel et al. 1998a; Jackson and Mantsch 2000; Wetzel and LeVine 2000; Dumas et al. 2004). The band at 1245 cm^{-1} represents the cellulosic compound (Figure 1 e). The assignments of frequently-used bands in life science application have been detailed and reviewed (Wetzel et al. 1998a; Yu 2005b).



(a) Typical DRIFT spectrum in the region: ca 4000-800 cm^{-1}

(c) Aromatic lignin peak area (Region: ca. 1536-1484 cm^{-1})

(e) Cellulosic compound peak area (Region: ca.1293-1212 cm^{-1})



(b) Molecular FTIR Spectrum in the region: 1800-800 cm^{-1}

(d) Total carbohydrate peak area (Region: ca.1192-932 cm^{-1})

Figure 2 Typical FTIR spectrum of barley hull: (a) whole mid-IR region: ca. 4000-800 cm^{-1}; (b) Fingerprint region: ca. 1800-800 cm^{-1}; (c) Aromatic lignin peak area: ca. 1510 cm^{-1}; (d) Total carbohydrate peak area region: ca. 1192-932 cm^{-1}; (e) Cellulosic compound peak area: ca 1245 cm^{-1}.

The characteristic IR absorption parameters obtained for hull samples are given in Table . Since less non-structural CHO and protein are contained in hull samples, lignin and cellulosic compounds were more concentrated. As expected, the results showed significant differences in the peak area of aromatic lignin, cellulosic compound, total CHO, and the ratio of lignin to cellulosic compound. The results indicate the obvious presence of lignin in the samples due to the aromatic characteristic peak at 1510 cm^{-1}. A high percentage of lignin may negatively affect the digestibility of the seed for ruminants. CDC Helgason had the largest ($P<0.05$) absorption peak area (2.51), implying a relatively higher concentration of lignin compared to the other barley varieties. This may be responsible for the slower degradation rate exhibited in *in situ* experiment. CDC Dolly had the smallest ($P<0.05$) lignin peak area (1.91) among the six barley varieties. The remaining four varieties were moderate from 1.99 to 2.26. The cellulosic absorption band at 1245 cm^{-1}, was also different between varieties (Table 1). The cellulosic compound peak area of CDC Helgason and CDC Cowboy was 1.75 and 1.66, respectively. The other varieties showed lower band intensity (from 1.18 to 1.53) of cellulosic material. In this study, the CHO absorption peak was defined at the region from 1192 to 932 cm^{-1}. CDC Helgason had a distinct higher ($P<0.05$) value (32.30) compared with other five varieties, which varied from 19.68 to 25.86.

Table 1. The structural characteristics of aromatic lignin, cellulosic compound and total carbohydrate and their ratios in the barley hull, revealed using DRIFT spectroscopy: Comparison of six barley varieties (n = 10 samples per each type of barley hull).

Items	Peak center (cm^{-1})	Region (cm^{-1})	Baseline (cm^{-1})	Molecular characteristics of barley hull in terms of the mid-IR absorbed peak area and their ratios (Infrared absorbed intensity unit)						SEM^z	P value
				AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy		
				----------Based on the peak area----------							
Aromatic lignin peak area	~1510	1536-1484	1536-1484	2.26 ab	1.91 c	1.99 bc	2.51 a	2.06 bc	2.26 ab	0.097	0.0006
Cellulosic compound peak area	~1245	1293-1212	1293-1212	1.36 c	1.18 d	1.53 b	1.75 a	1.34 c	1.66 a	0.037	<0.001
Total carbohydrate (CHO) area	-	1192-932	1192-932	22.33 b	19.80 b	25.86 ab	32.30 a	19.68 b	22.26 ab	2.548	0.0087
				----------Based on the peak area ratio----------							
Ratio of Lignin to total CHO	-	-	-	0.12	0.12	0.11	0.08	0.18	0.43	0.126	0.5062
Ratio of Cellulosic to total CHO	-	-	-	0.07	0.07	0.08	0.06	0.12	0.25	0.075	0.4900
Ratio of Lignin to Cellulosic	-	-	-	1.67 a	1.62 ab	1.30 d	1.43 bcd	1.54 abc	1.37 cd	0.072	0.0036

^{z} SEM = pooled standard error of means; Means with the different letter in the same row are significantly different ($P<0.05$). Mean separation was done by using the Fisher's protected LSD test method.

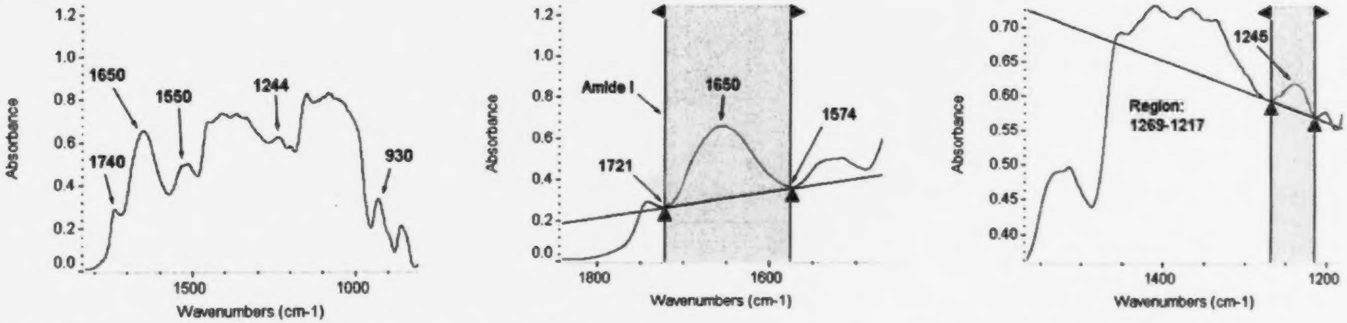
Cellulosic materials and lignin resist microbial breakdown (Nocek and Tamminga 1991). An earlier study showed that in contrast to hulless varieties, hulled barley had less soluble and degradable fractions, implying that the hull may be of importance in barley quality evaluation (Khorasani et al. 2000). As previously reported, most fibrous materials exist in the hull, which may also contribute to its poor digestibility (Bowman et al. 2001). Due to the presence of lignin, feedstuffs may more resistant to digestion in the rumen and lower digestive tract than that with less lignin (Tamminga et al. 1994). Cellulosic compounds also contribute to the variation in digestibility in the rumen. It is therefore expected that cellulosic compounds from the hull may also contribute to the decreased digestion rate of this material. This may also partly explain the lower ruminal digestibility of CDC Helgason compared to the other five barley varieties. Resistant compounds, such as lignin and cellulosic materials that are resistant to digest, can negatively affect the feed digestibility in the rumen. CDC Dolly had a similar particle size (Mean: 3.09 mm; Median: 2.74 mm) than CDC Helgason (Mean: 3.09 mm; Median: 2.75 mm), but a faster degradation rate, which may be attributed to its lower lignin (1.91) and cellulosic compound (1.18) contents. This may also partly explain the slowest degradation rate and smallest EDST of CDC Helgason. The relevant nutrients compounds ratios (lignin to total CHO, cellulosic compounds to total CHO and lignin to cellulosic compounds) were also determined. The results are presented in Table . Ratio of lignin to CHO and cellulosic to CHO were not different ($P>0.05$) among the six barley varieties. However, the ratio of lignin: cellulosic differed ($P<0.01$) among the six barley varieties, varying from 1.30 to 1.67.

Differences in the spectral data are dependent on the chemical make-up of the biological tissue. The differences observed in this study indicate that the hull of different barley varieties vary in structural and chemical make-up. These differences may contribute to the diverse rumen degradation behaviours. Based on this study, it is reasonable to assume that CDC Helgason may have a slower rumen degradation rate than other five barley varieties in rumen due to relatively higher lignin and cellulosic contents in the hull. This may also contribute to the significantly low effectively degradability of DM, CP and ST of CDC Helgason determined in the animal trial. By far, there is no information available on the spectral information of barley hull. In order to provide more relevant information, the whole seed was used to differentiate the chemical make-up of these six barley varieties.

Using DRIFT Spectroscopy to Characterize and Compare Structure-Chemical Make-Up of Whole Seed Samples from the Six Barley Varieties

A typical spectrum from whole barley seed is shown in Figure 2. The amide I peak, centered at ca.1650 cm^{-1} (predominantly C=O stretching vibration), represents protein. The peak of cellulosic compounds appears at ca. 1246 cm^{-1}. The bands in the region of 1188-955 cm^{-1} accounts for the presence of total CHO.

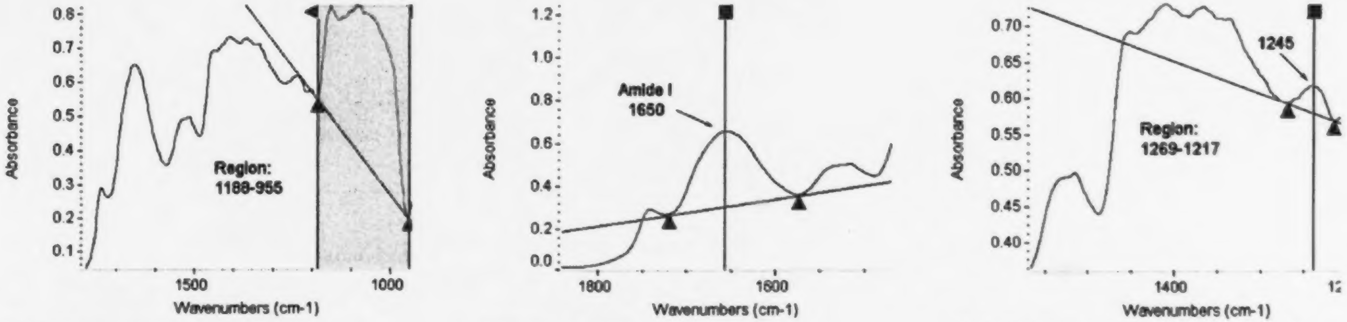
The peak absorption parameters from whole seed samples of barley are shown in Table 3. The protein components were examined by detecting characteristic bands caused by C=O stretching (amide I), C–N stretching and N–H bending (amide II) and O–C–N bending vibration. Relatively higher protein content in the biological tissue may be implied by the stronger IR absorption of amide I (ca. 1600-1700 cm^{-1}) and amide II (ca. 1500-1560 cm^{-1}) bands (Wetzel and LeVine 2001; Stuart 2004). In the amide I region (1721-1574 cm^{-1}), there were significant differences in band intensities. The amide I peak area data indicated that the whole seed sample of CDC Cowboy may contain the highest ($P<0.01$) concentration of protein (27.61). The amide I peak height data from the DRIFT spectra illustrated that the AC Metcalfe and CDC Cowboy had a relatively higher value (0.36 and 0.35, respectively), while CDC Helgason and CDC Trey were



(a) FTIR spectrum in the region: ca 1800-800 cm^{-1}

(c) Amide I peak area (Region: ca. 1721-1574 cm^{-1})

(e) Cellulosic compound peak area (Region: ca.1269-1217 cm^{-1})



(b) Total carbohydrate peak area (Region: ca.1188-955 cm^{-1})

(d) Amide I peak height (Region: ca. 1721-1574 cm^{-1})

(f) Cellulosic compound peak height (Region: ca.1269-1217 cm^{-1})

Figure 2. Typical FTIR spectrum of whole barley seed: (a) Fingerprint region: ca. 1800-800 cm^{-1}; (b) Total carbohydrate peak area region: ca. 1188-955 cm^{-1} (c,d) Amide I peak area and height: ca. 1650 cm^{-1}; (e,f) Cellulosic compound peak area and height: ca 1245 cm^{-1}.

Table 3. The structural characteristics of protein amide I and II, carbohydrates, structural carbohydrate (cellulosic compound) and their ratios in the whole barley seed, revealed using DRIFT spectroscopy: Comparison of six barley varieties (n = 10 samples per each type of whole barley seed).

Items	Peak center (cm^{-1})	Region (cm^{-1})	Baseline (cm^{-1})	Molecular characteristics of whole barley seed in terms of the mid-IR absorbed peak area and their ratios (Infrared absorbed intensity unit)						SEM^z	P value
				AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy		
				----------Based on the amide I peak area and height----------							
Amide I peak area	~1650	1721-1574	1721-1574	24.91 b	23.77 c	23.44 c	23.95 c	24.04 c	27.61 a	0.222	<0.0001
Amide I peak height	~1650	1721-1574	1721-1574	0.36 a	0.35 b	0.33 c	0.30 d	0.30 d	0.35 ab	0.003	<0.0001
				----------Based on the carbohydrate peak area----------							
Total Carbohydrate (CHO)	-	1188-955	1188-955	63.69 b	62.90 b	58.49 c	54.83 d	59.21 c	70.69 a	1.120	<0.0001
Structural CHO (cellulosic)	~1246	1269-1217	1269-1217	0.97 ab	0.95 bc	0.92 cd	0.84 e	0.88 de	1.00 a	0.015	<0.0001
				----------Based on the amide I and CHO peak area----------							
Ratio of Amide I to CHO area	1721-1574 / 1188-955		-	0.39 bc	0.38 c	0.40 b	0.44 a	0.41 b	0.39 bc	0.007	<0.0001
Ratio of total CHO to Structural CHO	1188-955/1269-1217		-	65.52 bc	66.16 bc	63.75 c	65.21 bc	67.28 ab	70.50 a	1.142	0.0031

^{z} SEM = pooled standard error of means; Means with the different letter in the same column are significantly different (P<0.05). Mean separation was done by using the Fisher's protected LSD test method.

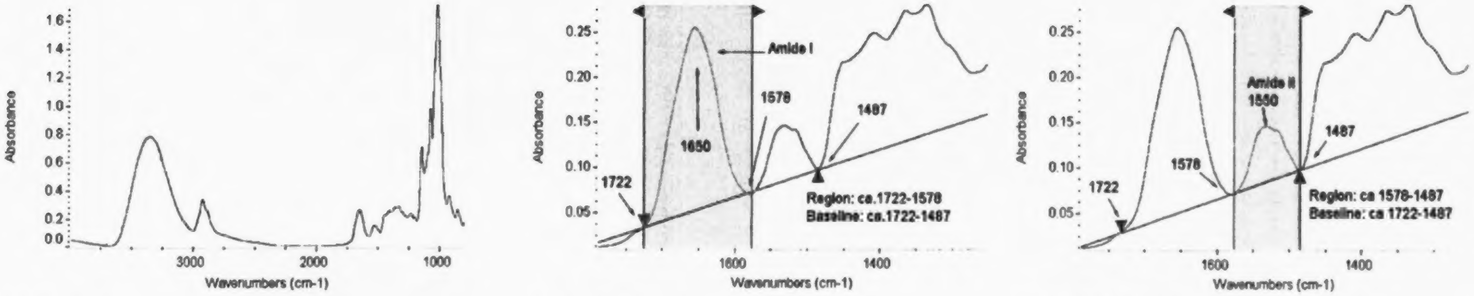
In addition, Table 3 shows that there was a significant difference in the CHO spectra absorbance of the barley varieties, with the CDC Cowboy having the highest ($P<0.05$) absorbance value of carbohydrate (70.69), while CDC Helgason had the lowest (54.83). The other four varieties were intermediate, ranging from 58.49 to 63.69. Similarly, for the structural CHO (cellulosic), CDC Cowboy and AC Metcalfe revealed a relatively higher peak height (1.00 and 0.97, respectively). CDC Helgason and CDC Trey exhibited lower ($P< 0.05$) values (0.84 and 0.88, respectively). The results indicate that CDC Helgason was found to have a relatively higher CHO content in the hull. However, in the whole seed sample, CDC Helgason had the lowest total CHO peak intensity, indicating there was a higher concentration of CHO existed in barley hull. This leads to the conclusion that CDC Helgason has the lowest CHO in whole seed, with a large portion of CHO in hull, may contribute to its lowest degradation rate and extent compared to other five barley varieties.

Amide I to CHO area ratio is given in Table 3. CDC Helgason showed the highest ($P< 0.01$) ratio. Amide I absorption peak contributes to evaluating protein, thus, it is possible to assume that there is more protein surrounding CHO in CDC Helgason to protect against the degradation in rumen. This may also partially explain the lowest EDCP (Table 3) and EDST (Table 4) of CDC Helgason. The ratio of total CHO to structural CHO (cellulosic) was also calculated and significant differences were revealed among the six barley varieties (Table 2). CDC Cowboy had the highest ($P< 0.05$) ratio compared with other five barley varieties, indicating that CDC Cowboy may contain a relatively higher percentage of cellulosic compounds in the seed structure. Barley varieties differ in type and composition of starch and non-starch polysaccharides (Holtekjøen et al. 2006). This property influences the barley quality in either malting or feeding industry. Type and amount of structural carbohydrate may affect site and extent of starch digestion (Nocek and Tamminga 1991). A high starch content may be associated with greater digestibility (Bowman et al. 2001). However, CDC Cowboy exhibited moderate digestibility of starch in *in situ* experiments. It is probably because of the starch distribution in the endosperm tissue. But comparative data are too limited to confirm this conclusion.

The adoption of DRIFT spectroscopy for examining the complex biopolymers of plant tissue provides advantages over traditional chemical analysis techniques. The DRIFT spectroscopy method has proven to be a powerful tool in terms of characterizing and discriminating structural and chemical differences of barley varieties. This technique may be used as an effective approach to rapidly differentiate and analyze of such samples.

Using Synchrotron-Based FTIR Microspectroscopy (SFTIRM) to Characterize and Compare Structural and Chemical Make-Up of Seed Endosperm Tissue among Six Barley Varieties

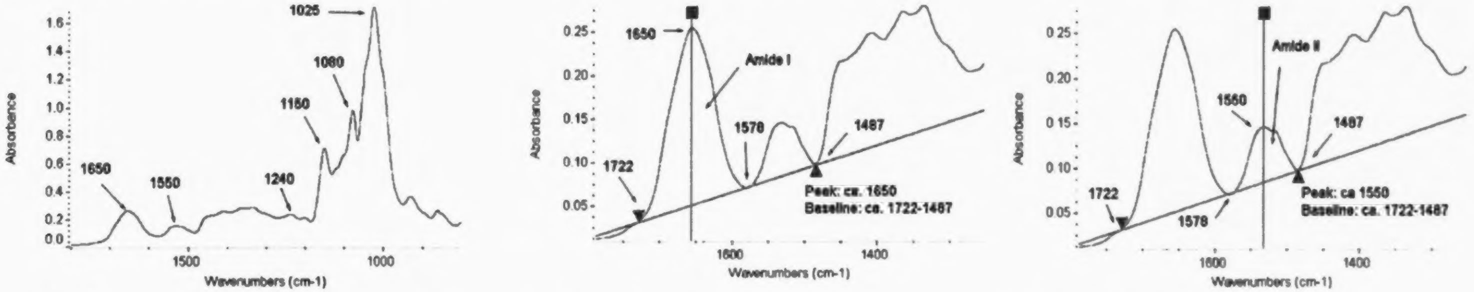
For SFTIRM, the barley seed specimens were optically observed with the coupled microscope (Figure A4) and spot sampling was done randomly on endosperm tissue (between 100-600 μm from outside of the seed section) of each barley variety to produce the spectral data. Figure 3 (a) illustrates the typical SFTIR spectrum of the endosperm tissue in the region of ca. 4000-800 cm^{-1}. The IR spectra of characteristic bands associated with specific nutrients (protein, cellulosic compound in Figure 3 and carbohydrates in Figure 4) are shown as well.



(a) Typical synchrotron-based FTIR spectrum in the region: ca 4000-800 cm^{-1} in the endosperm tissue

(c) Amide I peak area (Region: ca. 1722-1587 cm^{-1})

(e) Amide II peak area (Region: ca.1587-1487 cm^{-1})

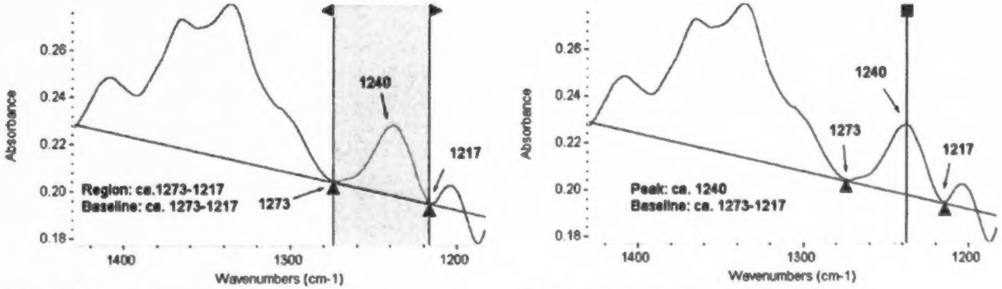


(b) FTIR spectrum in the fingerprint region: ca 1800-800 cm^{-1}

(d) Amide I peak height (Region: ca. 1722-1578 cm^{-1})

(f) Amide II peak height (Region: ca. 1587-1487 cm^{-1})

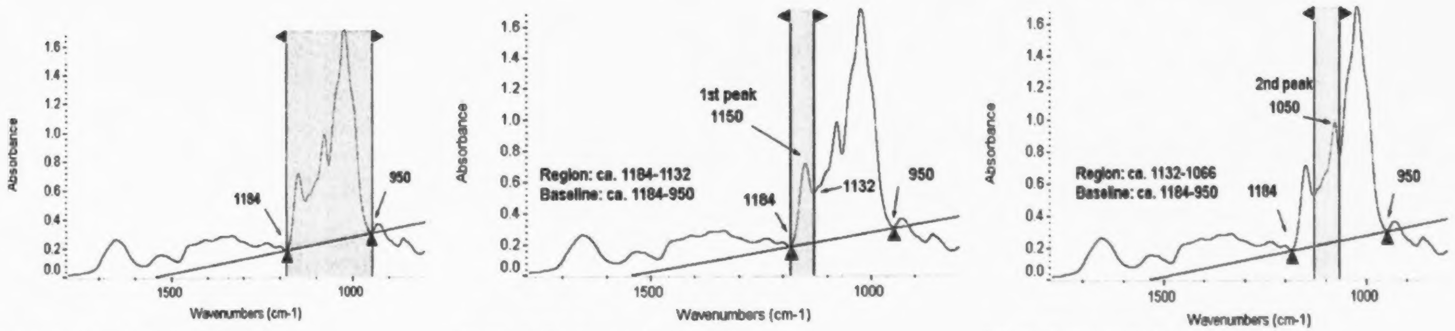
Figure 0. Typical synchrotron-based FTIR spectrum of endosperm tissue within a cellular dimension: (a) Whole mid-IR region: ca. 4000-800 cm^{-1}; (b) Fingerprint region: ca. 1800-800 cm^{-1}; (c,d) Amide I peak area and height: ca. 1650 cm^{-1}; (e,f) Amide II peak area and height: ca. 1650 cm^{-1}; (e,f) Amide II peak area and height: ca. 1550 cm^{-1}; (g, h) Cellulosic material peak area and height: ca 1240 cm^{-1};



(g) Cellulosic compound peak area
(Region: ca.1273-1217 cm^{-1})

(h) Cellulosic compound peak height
(Region: ca.1273-1217 cm^{-1})

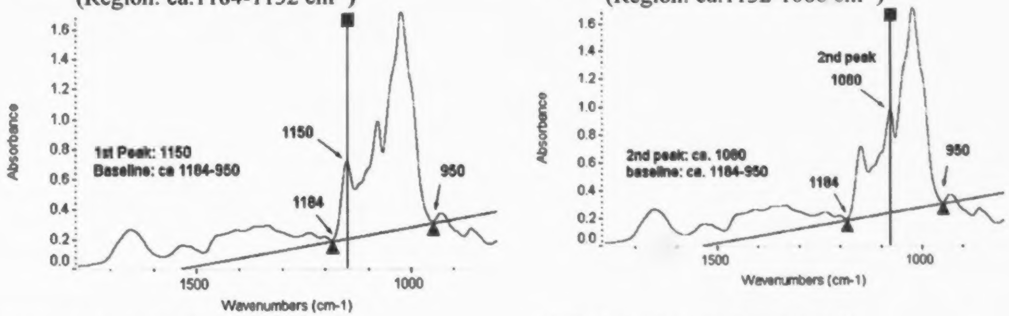
Figure 0. (continued) Typical synchrotron-based FTIR spectrum of endosperm tissue within a cellular dimension: (a) Whole mid-IR region: ca. 4000-800 cm^{-1}; (b) Fingerprint region: ca. 1800-800 cm^{-1}; (c,d) Amide I peak area and height: ca. 1650 cm^{-1}; (e,f) Amide II peak area and height: ca. 1650 cm^{-1}; (e,f) Amide II peak area and height: ca. 1550 cm^{-1}; (g, h) Cellulosic material peak area and height: ca 1240 cm^{-1}.



(a) Total carbohydrate peak area
(Region: ca.1184-950 cm^{-1})

(b) Carbohydrate 1st peak area
(Region: ca.1184-1132 cm^{-1})

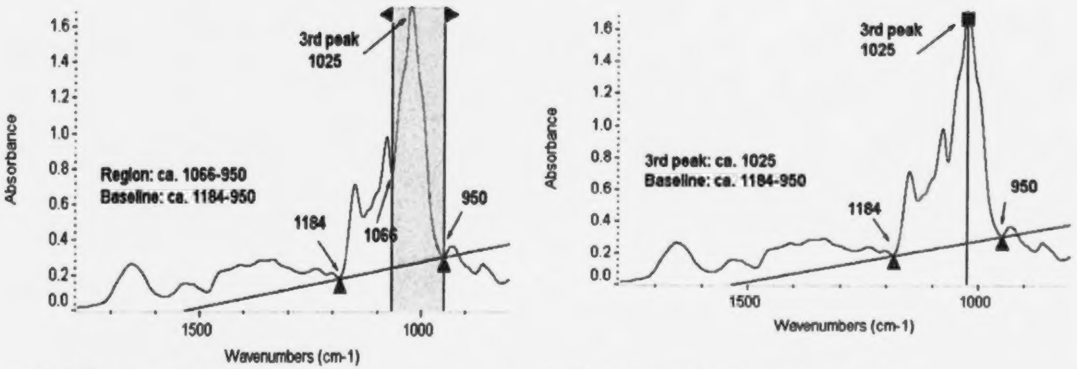
(d) Carbohydrate 2nd peak area
(Region: ca.1132-1066 cm^{-1})



(c) Carbohydrate 1st peak height
(Region: ca.1184-1132 cm^{-1})

(e) Carbohydrate 2nd peak height
(Region: ca.1132-1066 cm^{-1}

Figure 3 Typical synchrotron-based FTIR spectrum of endosperm tissue within a cellular dimension: (a) Total carbohydrate peak area region: ca. 1184-950 cm-1; (b,c) Carbohydrate 1st component peak area and height: ca. 1150 cm-1; (d,e) Carbohydrate 2nd component peak area and height: ca. 1080 cm^{-1}; (f,g) carbohydrate 3rd component peak area and height: ca. 1025 cm^{-1}.



(f) Carbohydrate 3rd peak area
(Region: ca. 1066-950 cm^{-1})

(g) Carbohydrate 3rd peak height
(Region: ca. 1066-950 cm^{-1})

Figure 4 (continued) Typical synchrotron-based FTIR spectrum of endosperm tissue within a cellular dimension: (a) Total carbohydrate peak area region: ca. 1184-950 cm^{-1}; (b,c) Carbohydrate 1st component peak area and height: ca. 1150 cm^{-1}; (d,e) Carbohydrate 2nd component peak area and height: ca. 1080 cm^{-1}; (f,g) carbohydrate 3rd component peak area and height: ca. 1025 cm^{-1}.

The characteristic absorbance bands of the amide groups can be used to probe and characterize the structural and chemical make-up of the plant tissue. The amide I (ca. 1650 cm^{-1}) and amide II (ca. 1550 cm^{-1}) peak areas and height definition used for univariate analysis are shown in the Figure 3 (c, d, e, f). The peak centered at approximately 1240 cm^{-1} (Figure 3 g, h) was considered to represent the structural carbohydrates (cellulosic compounds) (Wetzel et al. 1998a; Stuart 2004; Yu 2005b). The total carbohydrate peak area is located at region from ca.1184 to 951 cm^{-1} because of C-O and C-C stretching vibrations and C-O-H deformation (Wetzel et al. 1998a; Stuart 2004; Yu 2005b). The CHO band intensities were determined by calculating the peak heights and areas of specific bands: carbohydrate 1st peak area occurs at region of ca.1184-1132 cm^{-1}, carbohydrate 2nd peak area occurs at region of ca.1132-1066 cm^{-1} and carbohydrate 3rd peak area occurs at region of ca. 1066-950 cm^{-1}. These three peaks are regarded to represent total carbohydrate compounds (Figure 4).

To detect the chemical features associated with nutrients, synchrotron-based IR microspectroscopy was used to examine barley endosperm tissue. Spectroscopic data from spot samples within cellular dimensions are detailed in Table 4 to 5. It should be realized that the peak intensity parameters (area or height for instance) cannot be considered as representation of accurate biological compound contents.

Table 4. The structural characteristics of protein amides I and II in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy: Comparison of six barley varieties.

Items	Peak center (cm^{-1})	Region (cm^{-1})	Baseline (cm^{-1})	Molecular characteristics of whole barley seed in terms of the mid-IR absorbed peak area and their ratios (Infrared absorbed intensity unit)						SEM[z]	P value
				AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy		
Amide molecular spectral features				----------Based on the amides I and II peak area and height---------------							
Amide I peak area	~1650	1722-1578	1722-1487	7.99 bc	8.23 abc	10.02 a	7.33 c	9.01 ab	9.23 ab	0.582	0.004
Amide I peak height	~1650	1722-1578	1722-1487	0.11 cd	0.12 bcd	0.16 a	0.11 d	0.14 ab	0.13 bc	0.091	<0.0001
Amide II peak area	~1550	1578-1487	1722-1487	1.94 b	1.97 ab	2.57 a	1.86 b	2.20 ab	2.34 a	0.182	0.024
Amide I plus II area	-	1722-1578	1722-1487	9.93 bc	10.20 abc	12.59 a	9.19 c	11.22 ab	11.56 a	0.754	0.005

[z] SEM = pooled standard error of means; Means with the different letter in the same row are significantly different ($P<0.05$). Mean separation was done by using the Fisher's protected LSD test method.

Table 5 The structural characteristics of carbohydrates, and structural carbohydrate (cellulosic compounds) in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy: Comparison of six barley varieties.

Items	Peak center (cm^{-1})	Region (cm^{-1})	Baseline (cm^{-1})	Molecular characteristics of whole barley seed in terms of the mid-IR absorbed peak area and their ratios (Infrared absorbed intensity unit)						SEM[z]	P value
				AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy		
Carbohydrate (CHO) molecular spectral features				----------Based on the carbohydrate peak area and height----------							
Cellulosic compound area	~1240	1273-1217	1273-1217	0.54 a	0.53 ab	0.48 b	0.54 a	0.47 b	0.49 b	0.020	0.003
Total carbohydrate peak area	-	1184-951	1184-951	71.10 a	75.20 a	62.68 b	76.71 a	72.30 a	72.31 a	2.889	0.022
CHO: 1st component peak area	~1150	1184-1132	1184-951	8.25 b	8.67 ab	7.03 c	9.02 a	8.14 b	8.19 b	0.374	0.006
CHO: 1st component peak height	~1150	1184-1132	1184-951	0.29 a	0.30 a	0.24 b	0.27 ab	0.28 a	0.28 a	0.012	0.048
CHO: 2nd component peak area	~1080	1132-1066	1184-951	10.41 ab	11.24 a	9.54 b	11.14 a	10.74 ab	10.64 ab	0.460	0.175
CHO: 2nd component peak height	~1080	1132-1066	1184-951	0.42 ab	0.45 a	0.38 b	0.44 a	0.42 ab	0.42 ab	0.017	0.144
CHO: 3rd component peak area	~1025	1066-951	1184-951	44.92 b	47.38 ab	39.59 c	48.66 a	46.08 ab	46.30 ab	1.791	0.014
CHO: 3rd component peak height	~1025	1066-951	1184-951	0.66 ab	0.69 ab	0.59 b	0.72 a	0.72 a	0.72 a	0.029	0.014

[z] SEM = pooled standard error of means; Means with the different letter in the same row are significantly different ($P<0.05$). Mean separation was done by using the Fisher's protected LSD test method.

Table 6 The structural characteristics of the ratios of protein amide I and II, and structural (cellulosic compounds) and non-structural (NSC, starch) carbohydrates (CHO) in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy: Comparison of six barley varieties.

Items	Peaks area regions (baseline) (cm^{-1})	Molecular characteristics of whole barley seed in terms of the mid-IR absorbed peak area and their ratios (Infrared absorbed intensity unit)						SEM^z	P value
		AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy		
		--------Based on the Amide I and CHO peak area and height ratios --------							
Ratio of total CHO peak area / Amides I plus II peak area	1184-951 (baseline: 1184-951)/ 1722-1487 (baseline 1722-1487)	8.90 b	9.15 abc	7.11 c	9.89 ab	10.81 a	9.49 ab	0.618	0.002
Ratio of NSC (starch) peak area (CHO 3rd peak) / Amides I peak area	1066-951 (baseline:1184-951) / 1722-1487 (baseline 1722-1487)	6.91 b	6.86 abc	5.35 c	7.68 ab	8.25 a	7.58 ab	0.505	0.003
Ratio of NSC (starch) peak height (CHO 3rd peak) / Amide I height	~1025 (baseline: 1184-951) / ~1650 (baseline: 1722-1487)	6.91 bc	7.24 bc	5.16 c	7.90 b	8.41 ab	9.97 a	0.789	<0.001
Ratio of amides I & II peak area / cellulosic compounds peak area	1722-1487 (baseline: 1722-1487) / 1273-1217 (baseline: 1273-1217)	19.41 b	20.98 ab	26.44 a	17.99 b	23.64 a	24.29 a	1.568	<0.0001
Ratio of amide I peak area /cellulosic compounds peak area	1722-1587 (baseline: 1722-1487) / 1273-1217 (baseline: 1273-1217)	15.44 b	16.92 ab	21.03 a	14.27 b	18.96 a	19.36 a	1.190	<0.0001
Ratio of Amide I peak height /Cellulosic compounds peak height	~1650 (baseline: 1722-1487) / 1273-1217 (baseline: 1273-1217)	5.90 c	6.36 bc	9.77 a	5.67 c	7.87 b	7.67 b	0.501	<0.0001
Ratio of total CHO peak area / Cellulosic compound peak area	1184-951 (baseline: 1184-951)/ 1273-1217 (baseline: 1273-1217)	137.14 bc	144.80 abc	130.89 c	144.99 abc	157.57 a	148.60 ab	5.963	0.022
Ratio of NSC (starch) peak area (CHO 3rd peak) /Cellulosic compound peak area	1066-951 (baseline: 1184-951)/ /1273-1217 (baseline: 1273-1217)	87.08 bc	91.26 abc	82.65 c	92.71 abc	101.24 a	95.32 ab	3.936	0.011
Ratio of NSC (starch) peak height (CHO 3rd peak)/Cellulosic compound peak height	~1025 (baseline: 1184-951) / 1273-1217 (baseline: 1273-1217)	34.56 d	36.47 cd	35.82 cd	38.45 bc	42.47 a	40.93 ab	1.271	<0.0001

^{z}SEM = pooled standard error of means; Means with the different letter in the same row are significantly different ($P<0.05$). Mean separation was done by using the Fisher's protected LSD test method.

The amide bands representing protein at 1650 and 1550 cm^{-1} exhibited significant differences among the six barley varieties. Table 4 indicated that McLeod had the largest ($P<0.05$) amide I peak area (10.02), indicating a greater concentration of protein in the endosperm tissue. In contrast, CDC Helgason showed the lowest value (7.33). The other four values were in between. For amide I peak height value, the data varied significantly from 0.11 to 0.16. The peak areas and height centered at 1550 cm^{-1} (amide II) bands of McLeod exhibited the highest value (2.57, 0.05 respectively), again implying a greater concentration of protein in the endosperm tissue. By analyzing the peak of the total amides I and II area (Table 3), McLeod was still the greatest (12.59), while the CDC Helgason was the smallest (9.19). This may partially explain the lowest EDCP of CDC Helgason among the six barley varieties. If these six barley varieties are ranked based on their amide groups intensities, it is obvious that all the amide groups parameters show a similar trend that CDC Helgason exhibited the smallest value whereas McLeod showed the greatest peak intensity. The remaining four, in which AC Metcalfe and CDC Dolly had relatively smaller values, and CDC Trey and CDC Cowboy were relatively higher. These results also agree with the previous work that McLeod was developed as a high protein content barley (Camm 2008; Mikel and Kolb 2008). The protein may play a protector role if combined with carbohydrate, which may be related with the digestibility of barley.

Shown in Table 5 are the absorbance peak area and height data of non-structural and structural carbohydrates (cellulosic compounds) in the endosperm tissue of barley varieties. CDC Trey had the relatively smaller value (0.47) of the peak area at ca. 1240 cm^{-1} (cellulosic compounds band). In particular, there were differences found in the region around 1184-951 cm^{-1}, revealing the various CHO contents of the endosperm tissue of barley varieties. McLeod had the lowest ($P<0.05$) value of total carbohydrate peak area (62.68). The three characteristic absorption peaks fell in CHO region (1184-951 cm^{-1}) were analyzed respectively and it was observed similar trends. McLeod had the smallest ($P<0.01$) area of the 1st CHO peak (7.03), contrasting to the CDC Dolly and CDC Helgason (8.67 and 9.02, respectively). Compared to the other varieties, McLeod and CDC Helgason exhibited relatively lower ($P<0.05$) peak height of the 1st CHO peak (0.24 and 0.27, respectively). There was no significant ($P>0.05$) difference of 2nd CHO peak intensity shown here. At ca. 1066-951 cm^{-1}, there was an obvious absorption band which can be assigned to starch. The peak maxima appeared at ca. 1025 cm^{-1} (Wetzel et al. 1998a). Consistent with other CHO peak parameters, McLeod had the smallest ($P<0.05$) 3rd CHO peak area and relatively smaller peak height (0.59), suggesting the lowest enrichment of starch in endosperm tissue among the six barley varieties. Starch is abundant in endosperm tissue with different granule size, type and content (Takeda et al. 1999; You and Izydorczyk 2002). These variable molecular characteristics of barley endosperm tissue may have a strong influence on ruminal fermentation.

In order to extend the analysis of the structural and chemical make-up of the barley endosperm, relative intensity ratios of specific bands associated with nutrients were calculated. All items analyzed are presented in Table 6. The ratio analysis was calculated using absorbance intensity (area or height) of one characteristic peak observed in the spectrum to divide that of another one. McLeod had the relatively smaller ratio of total CHO peak area to amides I and II peak area (7.11). The ratio of 1205 cm^{-1} band assigned to starch: amide I band at 1650 cm^{-1} were significantly different among the six barley varieties. McLeod showed a relatively smaller value for both of the area and height ration comparison (5.35 and 5.16, respectively). According to the chemical composition analysis, McLeod has relatively more protein and less CHO content, which may be a reason of the smallest the CHO: amide ratio. Valier, another feed-type barley, was found to have lower starch: protein ratio in earlier study, implying that protein may protect starch from degradation in rumen (Yu 2006a).

There was also a notable difference in the ratio of the amide band to cellulosic compounds in the 1273-1217 cm^{-1} region. CDC Helgason, AC Metcalfe and CDC Dolly had relatively smaller ratio of amides I: cellulosic peak area (14.27, 15.44 and 16.92, respectively). The same trend was also found in peak area ratio comparison of amide I: cellulosic compounds. Whereas McLeod had the greatest ($P<0.05$) height ratio of amide I: cellulosic compounds (9.77). This is further evidence to support the opinion that cellulosic compounds significantly contribute to the lower digestibility.

Another large difference ($P<0.05$) was seen at peak area ratio of total CHO: cellulosic compounds. The higher starch content and lower undigestible fibrous materials may result in the better nutrients value, which has been noted in earlier study (Bowman et al. 2001). In comparison with CDC Trey (157.57) and CDC Cowboy (148.60), McLeod was merely 130.89. The ratio of non-structural CHO (starch) peak area (CHO 3rd peak): cellulosic compounds peak area exhibited a similar trend among these six barley varieties. CDC Trey and CDC Cowboy had a relatively higher ($P<0.05$) peak height ratio of non-structural CHO (starch) peak area (CHO 3rd peak): cellulosic compounds (42.47 and 40.93, respectively). Others were ranging from 34.56 to 38.45.

The protein matrix provides adhesion for starch granules in endosperm tissue (Holopainen et al. 2005). Cellulosic compounds in cell wall also take effort to support and maintain microstructure of seed. This association may influence the hardness of cereal, the particle size distribution, and as a result, nutritional value. With protein encompassing the starch granules, cultivars containing more protein may be associated with a harder structure. The protein content, structure, distribution and conjunction may partially explain the grain characteristics. Whereas, documents are lacking in individual spots sampling on barley grain using IR spectroscopic analysis. Little information is comparable. More study is needed to examine whether the spots sampling with Mid-IR spectroscopy can be used to predict nutrients availability of grain.

We can draw the conclusion that in conjunction with synchrotron light source, chemical-structural information can be obtained *in situ* via FTIR technique with intact tissue sections. Compared to traditional chemical analysis methodologies, for which grinding is always required, SFTIRM is obviously superior on probing the internal biological matrix with minimal artificial influence. Again, the results confirm earlier statements that with the advanced SFTIRM techniques, chemical-structural information of feedstuffs can be revealed. DRIFT data can be used to produce spectroscopic information of ground specimens, while the SFTIRM data can provide the ultrastructural-chemical make-up information of original biological tissue. Further research using SFTIRM techniques is needed since the results are limited.

Correlations between Mean and Median Particle Sizes, *In Situ* Degradation Features of DM, CP, and Starch and Mid-IR Spectral Characteristics among Barley Varieties

To extend this research, the correlation procedure was used in an attempt to relate spectral information with ruminal degradation characteristics. Pearson correlation coefficients were computed using Proc Corr procedure of SAS package to investigate the relationship between spectral data (from DRIFT and SFTRM) and degradation data (degradation rate and extent of DM, CP and ST in Table 6 to 10; S and D of DM, CP and ST in Table 11) for nutrient availability evaluation. The results displayed that the DRIFT spectroscopic data from hull samples were weakly correlated with the degradation characteristics (Table 6). Ratio of cellulosic to CHO was correlated with EDDM (P=0.082) and EDST (P=0.111). Aromatic lignin peak area was correlated with Kd of CP (P=0.082). However, no significant correlation was detected between the spectral data from whole seed sample and degradation rate and extent of DM, CP, and ST (Table 7). The correlation tendency also appeared between SFTIR information and degradation features, though not all of them were significant (Tables 8 to 11). CHO: 1st component peak area was correlated with Kd of CP (P=0.101) (Table 9). Cellulosic compound area was correlated with S (%) of CP (P=0.068) and S (%) of ST (P=0.041) (Table 11). More spectroscopic analysis on seed tissues is necessary to clarify this assumption that whether the chemical-structural make-up of endosperm tissue is linked to degradation performance of grain.

Weak correlations indicate that six barley varieties might not be sufficient enough to interpret the relationship between spectroscopic information and nutrients value of barley grain. In this case, it may due to the similarities and limitations of the barley varieties (only six barley varieties were examined). Differences among the varieties may not be large enough to reveal the actual relationship between the spectroscopic data and ruminal degradation data. Chemical and physical composition of plant tissue may affect the digestion process, but relevant reports referring to mid-IR spectroscopy have not been well documented yet. We aim at a further understanding of the relationship between IR spectroscopic information and nutrition value of feedstuffs. More studies involving a diverse range of samples with biological differences may thereby be necessary.

Table 6. Pearson correlation results between structural characteristics from hull sample and *in situ* rumen degradation rate and extent of six barley varieties. (The structural characteristics include aromatic lignin, cellulosic compound and total carbohydrate and their ratios in the barley hull, revealed using DRIFT spectroscopy).

Items	In situ rumen degradation kinetics of DM [z]				In situ rumen degradation kinetics of CP [z]				In situ rumen degradation kinetics of ST [z]			
	Kd (%/h)		EDDM (%)		Kd (%/h)		EDCP (%)		Kd (%/h)		EDST (%)	
	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Aromatic lignin peak area	-0.557	0.251	-0.305	0.556	-0.757	0.082	-0.358	0.486	-0.251	0.632	0.139	0.793
Cellulosic compound peak area	-0.501	0.311	-0.309	0.551	-0.644	0.167	-0.360	0.483	-0.365	0.477	0.232	0.659
Total carbohydrate (CHO) area	-0.670	0.145	-0.633	0.177	-0.701	0.121	-0.647	0.165	-0.576	0.232	0.576	0.231
Ratio of Lignin to CHO	0.503	0.309	0.440	0.382	0.655	0.158	0.477	0.339	0.371	0.469	-0.336	0.515
Ratio of Cellulosic to CHO	0.616	0.193	0.756	0.082	0.550	0.258	0.731	0.099	0.574	0.234	-0.714	0.111
Ratio of Lignin to Cellulosic	0.176	0.739	0.141	0.790	0.185	0.725	0.163	0.758	0.285	0.584	-0.199	0.706

[z] Kd = degradation rate; EDDM, EDCP, EDST= effective degradability of DM, CP and starch, respectively.

Table 7. Pearson correlation results between structural characteristics from whole seed sample and *in situ* rumen degradation rate and extent of six barley varieties. [The structural characteristics include protein amide I and II, carbohydrates, structural carbohydrate (cellulosic compound) and their ratios in the whole barley seed, revealed using DRIFT spectroscopy.]

Items	In situ rumen degradation kinetics of DM [z]				In situ rumen degradation kinetics of CP [z]				In situ rumen degradation kinetics of ST [z]			
	Kd (%/h)		EDDM (%)		Kd (%/h)		EDCP (%)		Kd (%/h)		EDST (%)	
	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Amide I peak area	0.021	0.969	0.251	0.631	-0.207	0.694	0.189	0.719	0.192	0.715	0.348	0.500
Amide I peak height	0.356	0.489	0.205	0.697	0.266	0.610	0.215	0.682	0.364	0.478	0.318	0.539
Amide I & II area	0.110	0.835	0.205	0.697	-0.118	0.825	0.165	0.754	0.285	0.584	0.388	0.418
Total Carbohydrate (CHO)	0.296	0.569	0.372	0.468	0.145	0.785	0.339	0.511	0.352	0.494	0.440	0.382
Structural CHO (cellulosic)	0.448	0.373	0.392	0.442	0.325	0.529	0.384	0.453	0.453	0.367	0.412	0.416
Ratio of Amide I to CHO area	-0.580	0.228	-0.410	0.419	-0.583	0.224	-0.432	0.393	-0.461	0.358	-0.379	0.459
Ratio of Amide I & II to CHO	-0.559	0.248	-0.518	0.292	-0.622	0.187	-0.524	0.286	-0.367	0.474	-0.302	0.561
Ratio of Structural to CHO	-0.224	0.670	-0.516	0.295	-0.127	0.811	-0.464	0.354	-0.275	0.598	-0.416	0.412

[z] Kd = degradation rate; EDDM, EDCP, EDST= effective degradability of DM, CP and starch, respectively.

Table 8. Pearson correlation results between structural characteristics from seed endosperm tissue and *in situ* rumen degradation rate and extent of six barley varieties. (The structural characteristics include protein amides I and II in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy.)

Items	In situ rumen degradation kinetics of DM [z]				In situ rumen degradation kinetics of CP [z]				In situ rumen degradation kinetics of ST [z]			
	Kd (%/h)		EDDM (%)		Kd (%/h)		EDCP (%)		Kd (%/h)		EDST (%)	
	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Amide I peak area	0.610	0.198	0.529	0.281	0.644	0.168	0.538	0.271	0.381	0.456	0.394	0.440
Amide I peak height	0.569	0.239	0.463	0.355	0.625	0.185	0.480	0.335	0.330	0.523	0.317	0.540
Amide II peak area	0.498	0.315	0.434	0.390	0.513	0.298	0.437	0.386	0.290	0.578	0.312	0.547
Amide II peak height	0.513	0.298	0.443	0.379	0.512	0.299	0.448	0.373	0.324	0.531	0.329	0.524
Amide I & II area	0.587	0.220	0.510	0.302	0.617	0.192	0.517	0.293	0.362	0.480	0.377	0.461

[z] Kd = degradation rate; EDDM, EDCP, EDST= effective degradability of DM, CP and starch, respectively.

Table 9. Pearson correlation results between structural characteristics from seed endosperm tissue and *in situ* rumen degradation rate and extent of six barley varieties. [The structural characteristics include carbohydrates and structural carbohydrate (cellulosic compounds) in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy.]

Items	In situ rumen degradation kinetics of DM [z]				In situ rumen degradation kinetics of CP [z]				In situ rumen degradation kinetics of ST [z]			
	Kd (%/h)		EDDM (%)		Kd (%/h)		EDCP (%)		Kd (%/h)		EDST (%)	
	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Cellulosic compound area	-0.511	0.300	-0.553	0.255	-0.560	0.247	-0.550	0.258	-0.321	0.535	-0.418	0.410
Cellulosic compound height	-0.349	0.497	-.0522	0.288	-0.257	0.623	-0.494	0.320	-0.347	0.500	-0.480	0.336
CHO: 1st component peak area	-0.711	0.113	-0.556	0.252	-0.728	0.101	-0.582	0.225	-0.515	0.296	-0.435	0.389
CHO: 1st component peak height	-0.021	0.968	0.058	0.914	-0.059	0.912	0.045	0.933	0.097	0.855	0.140	0.792
CHO: 2nd component peak area	-0.646	0.166	-0.505	0.307	-0.609	0.199	-0.528	0.282	-0.527	0.283	-0.426	0.399
CHO: 2nd component peak height	-0.630	0.180	-0.503	0.309	-0.610	0.199	-0.523	0.287	-0.484	0.331	-0.407	0.423
CHO: 3rd component peak area	-0.660	0.154	-0.449	0.372	-0.687	0.132	-0.486	0.329	-0.471	0.346	-0.336	0.515
CHO: 3rd component peak height	-0.509	0.302	-0.201	0.702	-0.579	0.229	-0.255	0.626	-0.313	0.546	-0.098	0.853
Total carbohydrate peak area	-0.668	0.147	-0.484	0.330	-0.680	0.137	-0.515	0.295	-0.487	0.327	-0.374	0.465

[z] Kd = degradation rate; EDDM, EDCP, EDST= effective degradability of DM, CP and starch, respectively.

Table 10. Pearson correlation results between structural characteristics ratios from seed endosperm tissue and *in situ* rumen degradation rate and extent of six barley varieties. [The structural characteristics include protein amide I and II, and structural (cellulosic compounds) and non-structural (NSC, starch) carbohydrates (CHO) in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy.]

Items	In situ rumen degradation kinetics of DM [z]				In situ rumen degradation kinetics of CP [z]				In situ rumen degradation kinetics of ST [z]			
	Kd (%/h)		EDDM (%)		Kd (%/h)		EDCP (%)		Kd (%/h)		EDST (%)	
	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Ratio of total CHO peak area / Amides I & II peak area	-0.279	0.593	0.030	0.955	-0.322	0.533	-0.012	0.982	-0.098	0.853	0.102	0.848
Ratio of NSC (starch) peak area (CHO 3rd peak) / Amides I peak area	-0.295	0.571	0.045	0.933	-0.380	0.457	-0.007	0.990	-0.081	0.879	0.138	0.795
Ratio of NSC (starch) peak height (CHO 3rd peak) / Amide I height	-0.294	0.572	0.042	0.937	-0.434	0.390	-0.026	0.961	-0.109	0.837	0.143	0.788
Ratio of amides I & II peak area / cellulosic compounds peak area	0.552	0.256	0.489	0.325	0.596	0.212	0.495	0.319	0.317	0.540	0.351	0.495
Ratio of amide I peak area / cellulosic compounds peak area	0.555	0.253	0.489	0.325	0.607	0.202	0.496	0.317	0.315	0.544	0.348	0.499
Ratio of Amide I peak height / Cellulosic compounds peak height	0.509	0.303	0.413	0.415	0.574	0.234	0.428	0.398	0.263	0.614	0.264	0.613
Ratio of total CHO peak area / Cellulosic compound peak area	-0.171	0.746	0.117	0.826	-0.175	0.741	0.076	0.887	-0.089	0.867	0.136	0.797
Ratio of NSC (starch) peak area (CHO 3rd peak) / Cellulosic compound peak area	-0.169	0.750	0.139	0.793	-0.190	0.718	0.094	0.860	-0.069	0.896	0.165	0.754
Ratio of NSC (starch) peak height (CHO 3rd peak) / Cellulosic compound peak height	-0.125	0.813	0.185	0.726	-0.155	0.770	0.134	0.801	-0.085	0.873	0.177	0.737

[z] Kd = degradation rate; EDDM, EDCP, EDST= effective degradability of DM, CP and starch, respectively.

Table 11. Pearson correlation results between structural characteristics from seed endosperm tissue and *in situ* rumen degradation characteristics of six barley varieties. [The structural characteristics include structural carbohydrate (cellulosic compounds) and amide I: cellulosic compound ratio in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy.]

	Degradation characteristics of DM[z]				Degradation characteristics of CP[z]				Degradation characteristics of ST[z]			
	S (%)		D (%)		S (%)		D (%)		S (%)		D (%)	
Items	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Cellulosic compound area	-0.614	0.195	-0.281	0.590	0.779	0.068	-0.507	0.304	0.830	0.041	-0.677	0.140
Cellulosic compound height	-0.527	0.283	-0.628	0.182	0.500	0.313	-0.823	0.044	0.404	0.427	-0.684	0.134
Ratio of amide I peak area /cellulosic compounds peak area	0.442	0.380	0.035	0.948	-0.648	0.164	0.213	0.685	-0.860	0.028	0.531	0.278

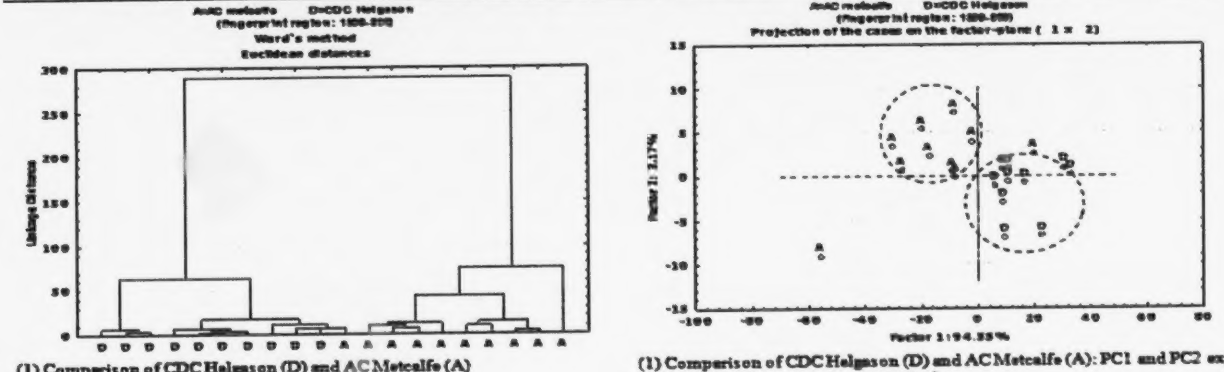
[z] S = soluble fraction; D= potentially degradable fraction of DM, CP and starch, respectively.

Using Mid-IR Spectroscopy with Multivariate Molecular Spectral Analysis to Discriminate and Classify Molecular Structure Difference of Barley Seed: Comparison among Barley Varieties

DRIFT technique was used to detect structural characteristics and identify the structural differences in chemical functional groups of the ground barley seed. Two multivariate analysis methodologies, AHCA and PCA were applied to the SFTIR spectroscopic data sets to organize the produced spectral data for comparison purposes. Since the fingerprint region can be used to identify the type of chemical compounds, primary interests were emphasized on the fingerprint region (1800-800 cm^{-1}) to conduct the multivariate spectral analyses. As the most distinctive variety, CDC Helgason was compared to other five varieties using AHCA and PCA analysis in attempt to access the attributes of varieties. The separation results are visually presented in Figure 5. We realized that the analysis results of the six barley varieties overlapped together. Therefore, in this study, the six barley varieties were contrasted to each other respectively to achieve the separation.

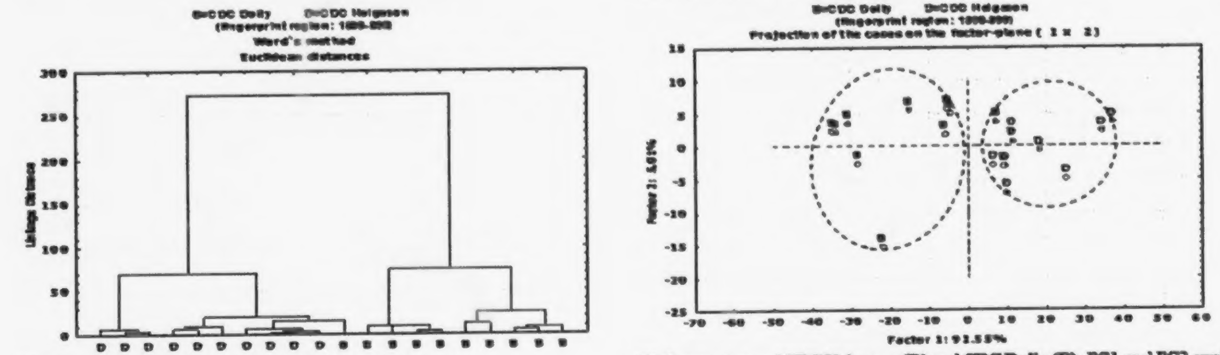
The AHCA analysis of the spectra of CDC Helgason compared with other five barley varieties was carried out and shown in Figure 5 (left column). Note that except CDC Trey, in other four paired comparisons, spectral data from different varieties can be obviously separated into two individual clusters within a linkage distance 100, implying that CDC Helgason has a different spectroscopic features in contrast with the other four varieties. However, the mixed dendrogram of CDC Helgason and CDC Trey showed similarities of spectral data in fingerprint region between these two varieties.

I: Cluster Analysis (CLA): Fingerprint region 1800-800 cm^{-1} (barely seed) II: Principal component analysis (PCA): Fingerprint region 1800-800 cm^{-1} (barely seed)



(1) Comparison of CDC Helgason (D) and AC Metcalfe (A)

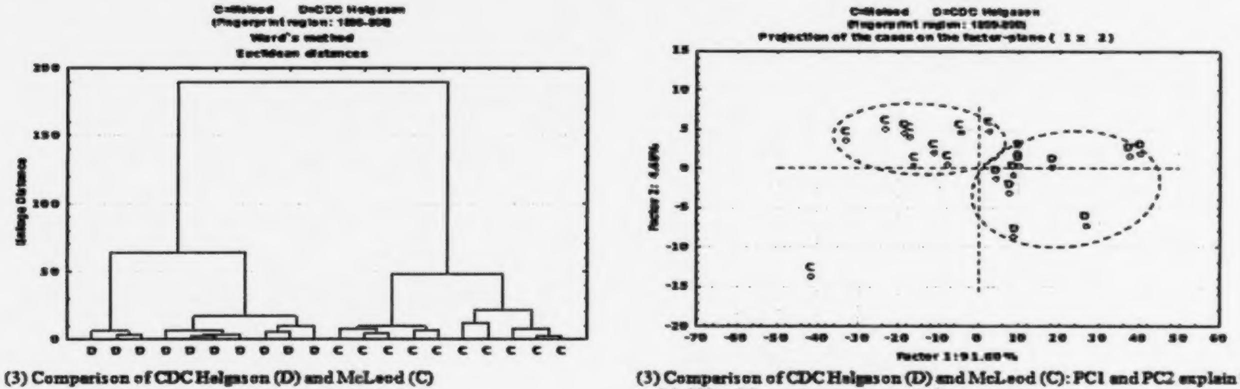
(1) Comparison of CDC Helgason (D) and AC Metcalfe (A): PC1 and PC2 explain 94.55 and 3.17% of the variances, respectively.



(2) Comparison of CDC Helgason (D) and CDC Dolly (B)

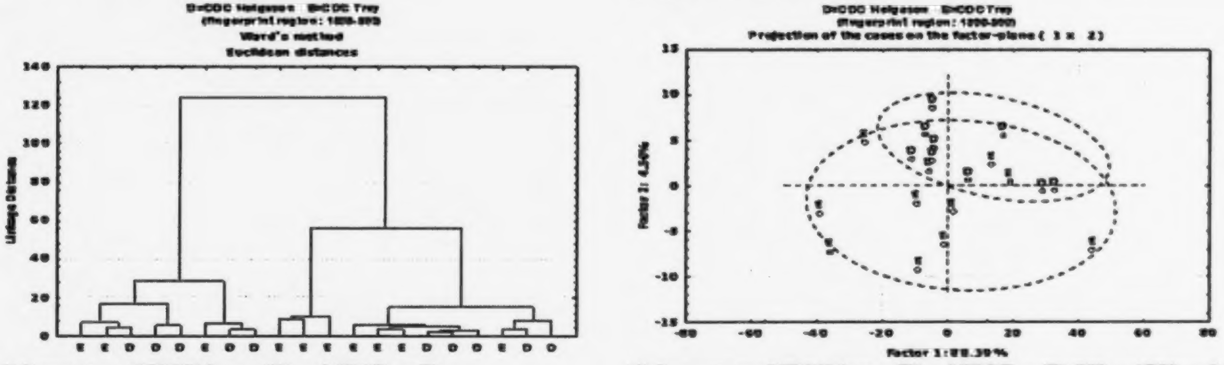
(2) Comparison of CDC Helgason (D) and CDC Dolly (B): PC1 and PC2 explain 91.55 and 5.01% of the variances, respectively.

Figure 5. Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgson (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral region: fingerprint region: ca. 1800 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).



(3) Comparison of CDC Helgason (D) and McLeod (C)

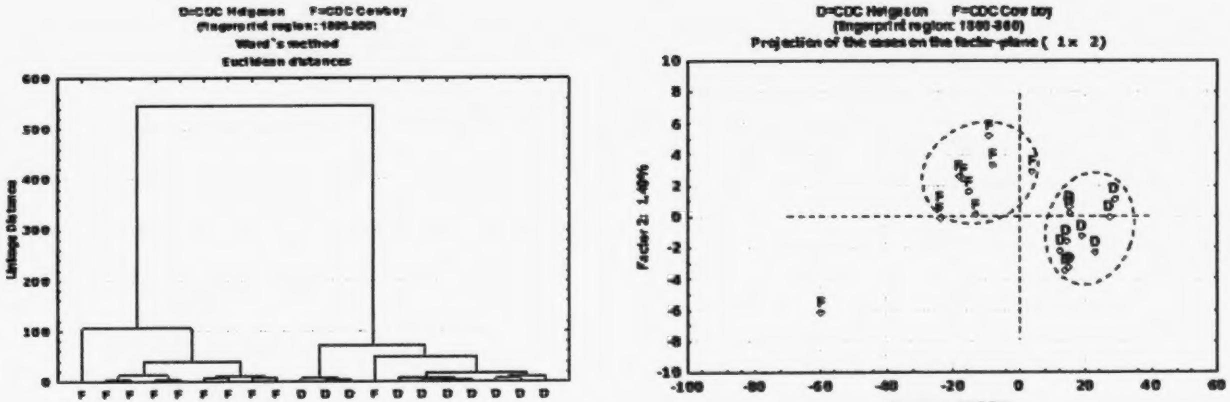
(3) Comparison of CDC Helgason (D) and McLeod (C): PC1 and PC2 explain 91.00 and 4.68% of the variances, respectively.



(4) Comparison of CDC Helgason (D) and CDC Trey (E)

(4) Comparison of CDC Helgason (D) and CDC Trey (E): PC1 and PC2 explain 88.30 and 4.56% of the variances, respectively.

Figure 5 (continued) Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgson (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral region: fingerprint region: ca. 1800 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).



(5) Comparison of CDC Helgason (D) and CDC Cowboy (F)

(5) Comparison of CDC Helgason (D) and CDC Cowboy (F): PC1 and PC2 explain 97.90 and 1.40% of the variances, respectively.

Figure 5 (continued) Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgson (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral region: fingerprint region: ca. 1800 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

PCA analysis results of CDC Helgason compared with other five barley varieties are shown as the 1st principal component (PC1) vs. the 2nd principal component (PC2) plot figure in Figure 5 (right column). The first two principal components explained predominant variation (88.30-97.90% for PC1 and 1.40-5.01% for PC2, respectively) of the spectral data. PCA analysis illustrates the same results as that from AHCA analysis, which clearly shows that CDC Helgason was distinguishable with AC Metcalfe, CDC Dolly, McLeod and CDC Cowboy in the PC1 vs. PC2 scatter plot. The barley varieties were grouped in different ellipses to detect the differences of chemical functional groups. It is possible to distinguish CDC Helgason from other barley varieties with the exception of CDC Trey. CDC Trey and CDC Helgason overlapped with each other, implying they have similar spectroscopic information, which may arise from their similarities of chemical and structural composition. Further study is necessary to detect the biological differences between CDC Helgason and CDC Trey.

The paired comparison also exhibited that CDC Cowboy could be clearly distinguished from AC Metcalfe, McLeod and CDC Trey in fingerprint region (see Appendix supplementary material Figure A1).

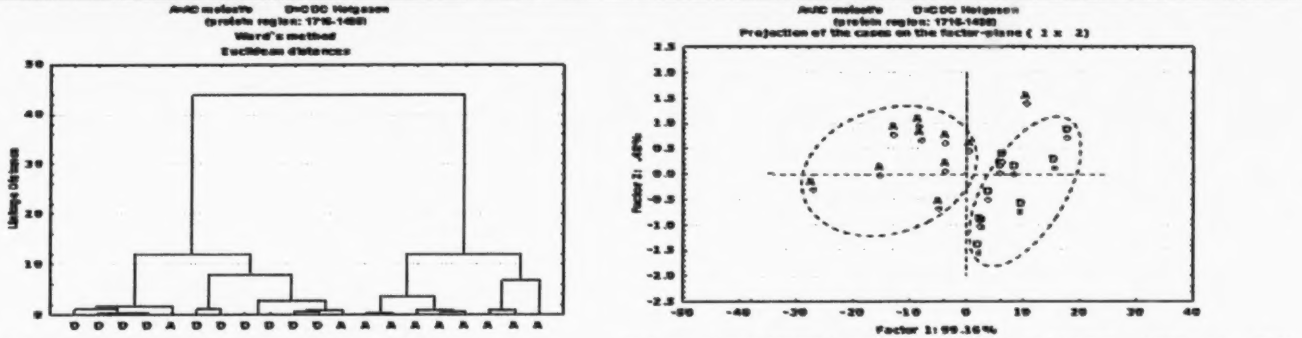
AHCA and PCA analysis provide statistical evidences that differences exist in chemical and structural conformation among the six barley varieties. This again supports our conclusion that CDC Helgason is particularly unique. Due to the insufficiency of related data from barley, more research is needed to make the classification and comparison among varieties to determine the biological effects on microstructure and other features associated with feed quality. As previously mentioned, in order to gain deeper insights of the chemical differences among these six barley varieties, attention was subsequently focused on the carbohydrates and protein. Comparisons, in collaboration of the multivariate methods, were also made in amide I and II region (1715-1485 cm^{-1}) and CHO region (1185-800 cm^{-1}).

The dendrograms and PCs scatter plots of barley varieties comparisons in protein (amide I and II) region are presented in Figure 6. Both the AHCA and PCA analysis illustrate similar results which are linked to the differences of spectroscopic information associated with chemical and physical properties of barley. As shown in Figure 6 (left column), the appearing two cluster indicate that the examined varieties have distinct spectroscopic features in amide I and II region. Except CDC Trey and CDC Dolly, CDC Helgason and other three barley varieties were well separated, forming two clusters.

This separation of varieties is more obviously exhibited in PCA analysis in Figure 6 (right column), shown as the scatter plots of the first two PCs. We note that different distribution in PCA plot represent different spectroscopic features linked to inhomogeneous conformation. This segregates varieties into different groups. Although CDC Helgason and CDC Trey form two individual ellipses in y-axis direction, the PC2 only explained 0.68% variation, which is not sufficient to demonstrate their dissimilarity in protein matrix conformation. Correspondingly, the results emphasize that CDC Helgason has thus been successfully differentiated from AC Metcalfe, CDC Dolly, McLeod and CDC Cowboy in amide I and II region, illustrating their dissimilarities in protein amide I and II region of 1715-1485 cm^{-1}. Additionally, CDC Cowboy and CDC Trey can be grouped into different family in protein region, indicating their differences in protein conformation (see Appendix supplementary material Figure A2).

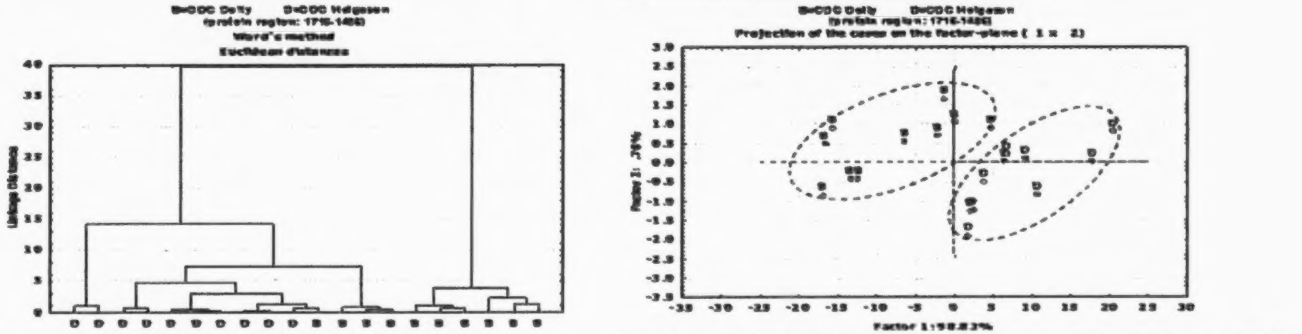
Whereas the relevant information is lacking, it is still difficult to conclude that CDC Helgason and CDC Trey have the same protein structural and chemical make-up. In addition, AHCA and PCA showed inconsistent results of comparison between CDC Dolly and CDC Helgason. An earlier study found that both the β-sheet and α-helices contribute to amide absorption respectively (Jackson and Mantsch 1991). More characterizing methods, such as FSD involving protein secondary structure analysis, may provide new angles on similarities and dissimilarities of chemical and structural information of protein matrix of barley grain. This information may be helpful for rapid evaluation of barley nutrient availability for ruminants.

I: Cluster Analysis (CLA): Fingerprint region 1715-1485 cm^{-1} (barely seed) II: Principal component analysis (PCA): Fingerprint region 1715-1485 cm^{-1} (barely seed)



(1) Comparison of CDC Helgason (D) and AC Metcalfe (A)

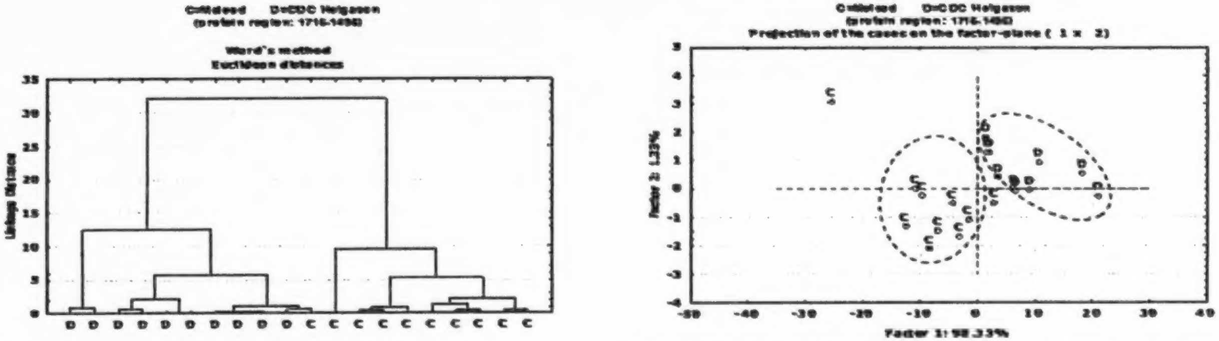
(1) Comparison of CDC Helgason (D) and AC Metcalfe (A): PC1 and PC2 explain 99.16 and 0.48% of the variances, respectively.



(2) Comparison of CDC Helgason (A) and CDC Dolly (B)

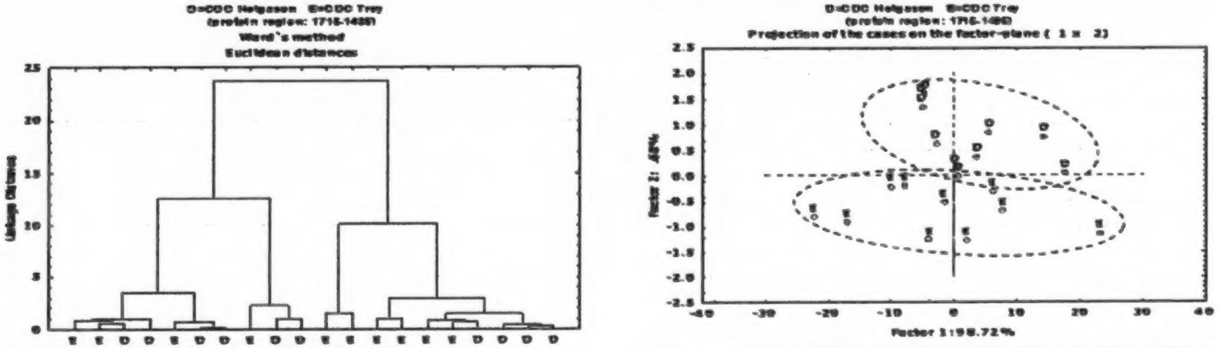
(2) Comparison of CDC Helgason (D) and CDC Dolly (B): PC1 and PC2 explain 98.83 and 0.70% of the variances, respectively.

Figure 6. Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgason (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral region: amides I and II region: ca. 1715 to 1485 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).



(3) Comparison of CDC Helgason (D) and McLeod (C)

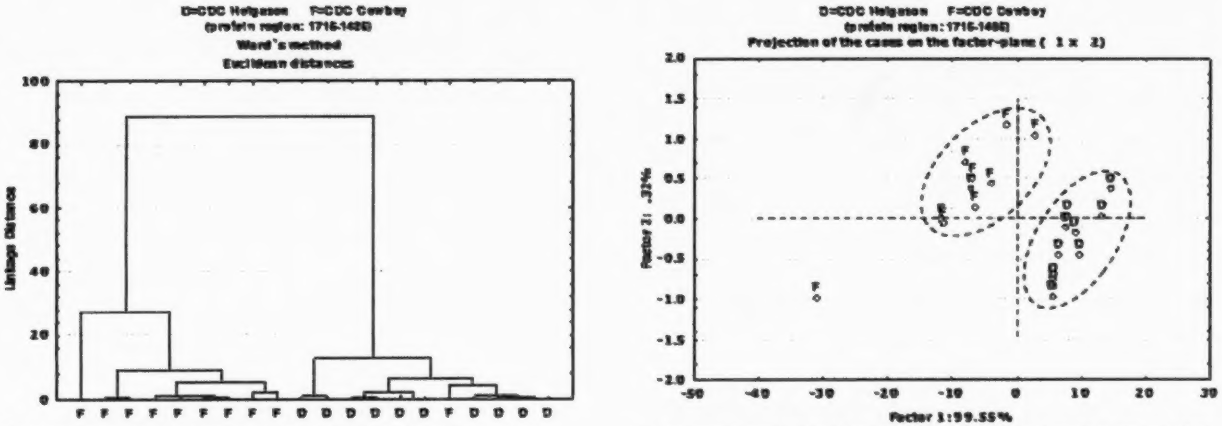
(3) Comparison of CDC Helgason (D) and McLeod (C): PC1 and PC2 explain 98.33 and 1.33% of the variances, respectively.



(4) Comparison of CDC Helgason (D) and CDC Trey (E)

(4) Comparison of CDC Helgason (D) and CDC Trey (E): PC1 and PC2 explain 98.72 and 0.68% of the variances, respectively.

Figure 6 (continued) Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgason (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral spectral region: amides I and II region: ca. 1715 to 1485 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).



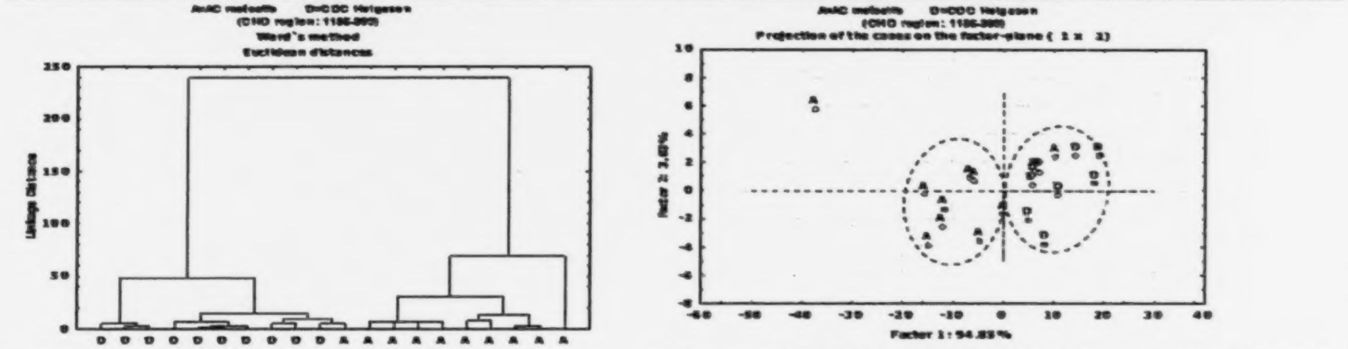
(5) Comparison of CDC Helgason (D) and CDC Cowboy (F)

(5) Comparison of CDC Helgason (D) and CDC Cowboy (F): PC1 and PC2 explain 99.55 and 0.32% of the variances, respectivaly.

Figure 6 (continued) Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgason (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral spectral region: amides I and II region: ca. 1715 to 1485 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

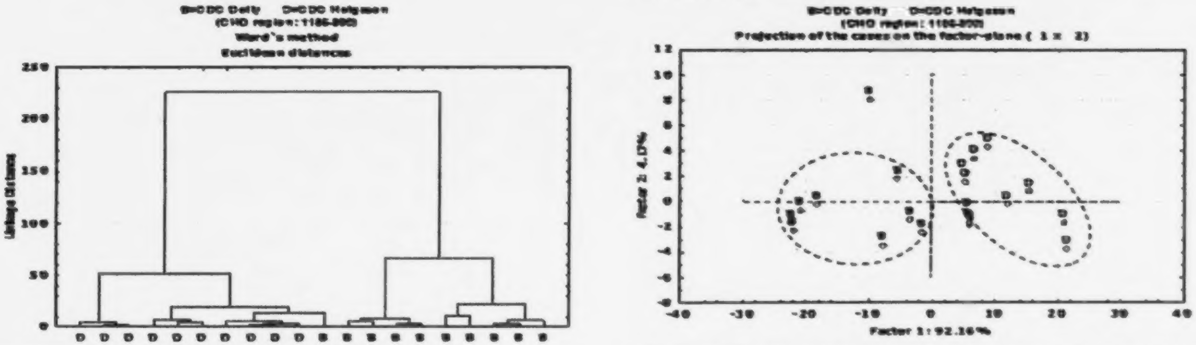
Comparisons were also made in spectral region assigned to CHO from 1185 to 800 cm^{-1} to further analyze the heterogeneity among the six varieties of the barley grain. As illustrated in Figure 7.7, the characteristic bands were examined as well using AHCA and PCA, two multivariate analysis methods. The dendrograms and the PCs scatter plots derived from the original spectral data graphically reveal the differences of structural and chemical make-up. Except CDC Trey, the uniqueness of CDC Helgason in structural and chemical differences was descriptive compared with other four barley varieties. CDC Trey and CDC Helgason could not be completely discriminated even though their degradation rate and extent of starch are significantly different. Presumably, CHO is more diverse than protein in barley, which is in agreement with the earlier study that barley varieties vary markedly in carbohydrates composition (Yu 2007a). Nevertheless, the information of CHO spectral difference using AHCA and PCA is completely lacking. No more references could be found to do further discussion.

By comparing the analysis results, variations were detected among different barley varieties, which evidenced that the protein amides I and II and/or CHO structural and chemical make-up properties of barley may account for the cultivar differences in digestibility for ruminants. The results indicated that the application of AHCA and PCA, two multivariate techniques, with the IR spectroscopy, makes it possible to efficiently discriminate and classify the inherent molecular structural features among the different barley varieties. These methods are especially helpful when unknown tissue is examined. Although the AC Metcalfe and CDC Dolly did not show significant differences using AHCA and PCA analysis of the original spectral data set in this study, another previous work (Yu 2007a) exhibited that these two barley varieties could be distinguished by applying PCA analysis with Fourier self-deconvolution (FSD) spectral data in amide I region (1710-1576 cm^{-1}), indicating more spectral analysis methodologies can be used to expand our understanding of the molecular information of the feed structure.



(1) Comparison of CDC Helgason (D) and AC Metcalfe (A)

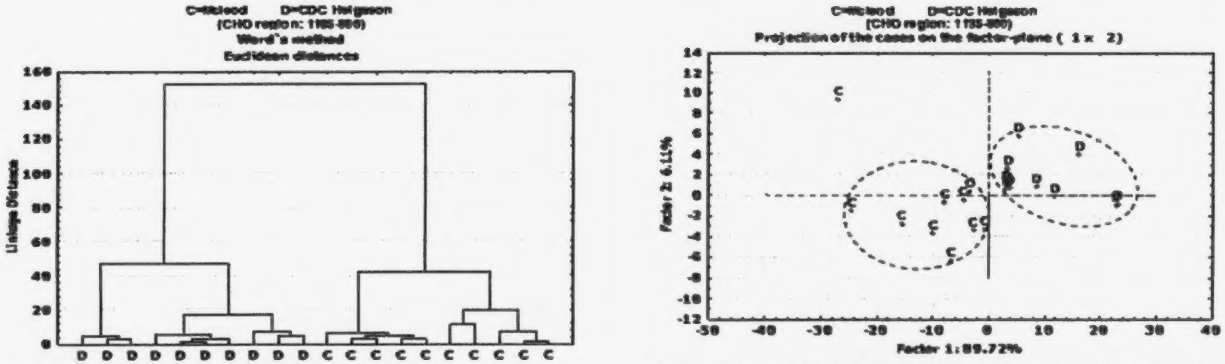
(1) Comparison of CDC Helgason (D) and AC Metcalfe (A): PC1 and PC2 explain 94.85 and 3.03% of the variances, respectively.



(2) Comparison of CDC Helgason (A) and CDC Dolly (B)

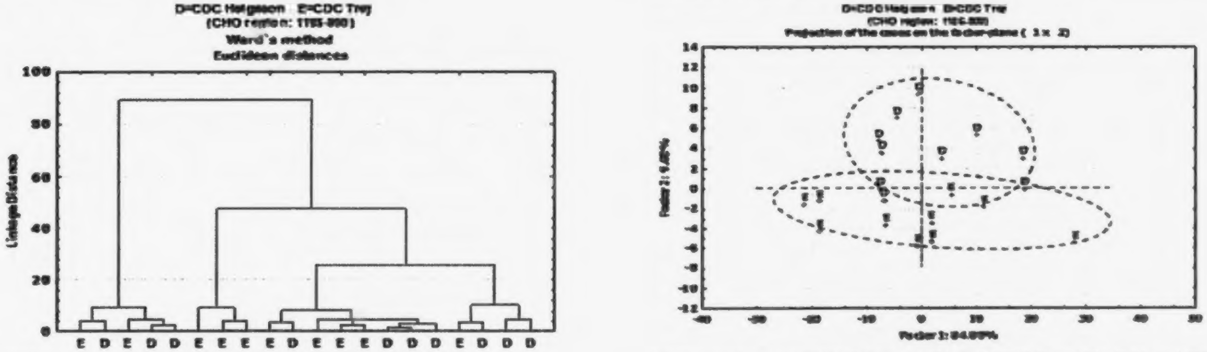
(2) Comparison of CDC Helgason (D) and CDC Dolly (B): PC1 and PC2 explain 92.16 and 4.17% of the variances, respectively.

Figure 7. Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgason (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral region: carbohydrate region: ca. 1185 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).



(3) Comparison of CDC Helgason (D) and McLeod (C)

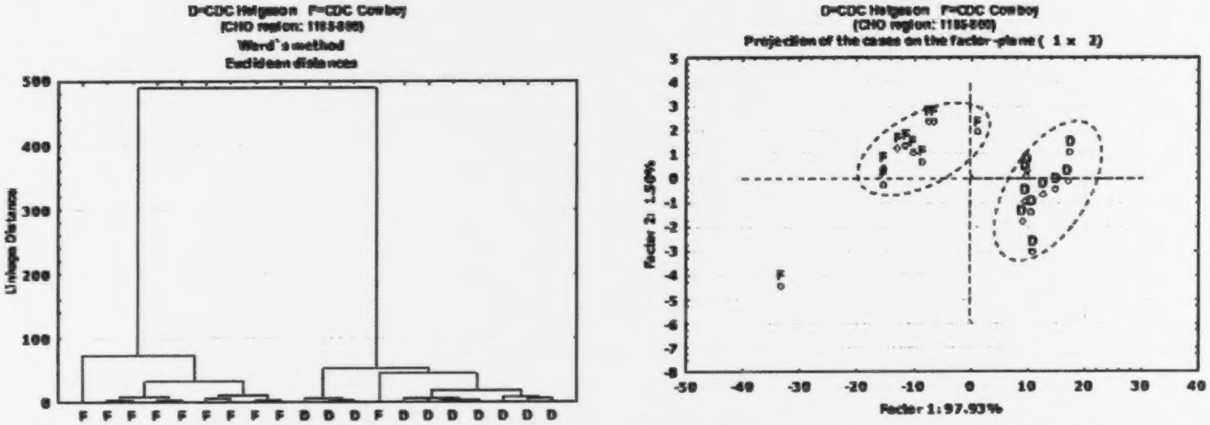
(3) Comparison of CDC Helgason (D) and McLeod (C): PC1 and PC2 explain 89.72 and 6.11% of the variances, respectively.



(4) Comparison of CDC Helgason (D) and CDC Trey (E)

(4) Comparison of CDC Helgason (D) and CDC Trey (E): PC1 and PC2 explain 84.00 and 9.60% of the variances, respectively.

Figure 7 (continued) Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgason (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral region: carbohydrate region: ca. 1185 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).



(5) Comparison of CDC Helgason (D) and CDC Cowboy (F)

(5) Comparison of CDC Helgason (D) and CDC Cowboy (F): PC1 and PC2 explain 97.93 and 1.50% of the variances, respectively.

Figure 7. (continued) Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgason (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral region: carbohydrate region: ca. 1185 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

Paired comparison of CDC Cowboy and four other varieties (AC Metcalfe, CDC Dolly, McLeod and CDC Trey) was computed. DRIFT spectral data of hull samples from six barley varieties and the SFTIRM data sets were also analyzed using these two multivariate methods. However, the results showed that it cannot be completely grouped in separate cluster (for AHCA) or ellipse (for PCA) because of overlapping of each paired comparison (data not shown). This may demonstrate that the microstructures of the hull and endosperm tissues from these barley varieties are quite similar to each other. Since this approach is so novel that few data have been reported, it should be pointed out that the structural information obtained from these two analysis methods is not the only determinant of cultivar variation. More study involving other structures of the barley kernel, such as pericarp or aleurone, may provide further insight into the varieties differences. Chemical mapping techniques may also provide further understanding on the similarities and dissimilarities.

Conclusions

Traditional chemical analysis methods are difficult to explore the structure or spatial conformation of feed samples. Failure to detect structural information and chemical distribution of complex biological organisms using traditional chemical analysis drives the development of novel research methodologies. DRIFT and SFTIRM techniques were used to investigate the structural and chemical features of barley varieties. The differences in chemical functional groups' characteristics, which are associated with feed nutrients, were determined in order to compare barley varieties. The results indicated that FTIR spectroscopy techniques could be applied to the detection of nutrients- related compounds that exist in barley grain. Therefore, the utilization of FTIR techniques for complex organic samples can be worthy to develop.

It is also conclusive that the differences in the chemical make-up features of whole seed and hull samples from the six barley varieties can be detected using DRIFT spectroscopy. The barley varieties can be differentiated with small sample volumes using this technique in comparison to traditional chemical analysis methodologies. The obtained results also indicated that the multivariate analysis methods (AHCA and PCA) can graphically distinguish structural and chemical differences with spectroscopic information. With these two multivariate spectral analysis techniques applied to the whole seed samples, CDC Helgason was distinguished from AC Metcalfe, CDC Dolly, McLeod and CDC Cowboy in the fingerprint (1800-800 cm^{-1}) and CHO region (1185-800 cm^{-1}), from AC Metcalfe, McLeod and CDC Cowboy in the protein region (1715-1485 cm^{-1}). However, CDC Helgason could not be distinguished from CDC Trey in either protein/CHO region or fingerprint region even though they exhibited significantly different degradation rate and extent using *in situ* technique. In addition, the results indicated that these six barley varieties may have similar structures of hull, but significant differences in whole seed.

Ultra-spatial resolved synchrotron IR microspectroscopy was used to monitor the intrinsic distribution of chemical compounds associated with nutrition in barley endosperm tissue. The informative results reflected that SFTIR spectroscopic analysis with excellent ultra-spatial resolution allows the determination of spectral data with structural and chemical information of the specimens in microscopic regions. In addition, the synchrotron light source is preferred because of its superior brightness and S/N ratio.

The different grain properties of barley varieties may arise from their heterogeneous nature of barley endosperm tissue. The structural differences among the barley varieties may be one reason of various digestive behaviours and nutritive quality to ruminants. Information from this study involving probing the seed internal structure of barley may provide a further insight as to why barley varieties exhibit different biodegradation behaviours. To provide an informative characterization of samples on both the feed quality evaluation and breeding selection, this technique is worth developing practically from theories.

Acknowledgements

The authors would like to thank Saskatchewan Agriculture Development Fund (ADF, Project # 20040495) for financing this project and Saskatchewan Agriculture Development Fund (ADF) for Ministry of Agriculture Strategic Feed Research Chair Program.

PART II: GENERAL DISCUSSION AND CONCLUSIONS

General Discussion and Conclusions

The results of this study show that there were significant differences in the mean and median particle size, and rumen degradation kinetics of each individual nutrient (DM, CP and starch) among the six barley varieties.

Based on the results obtained from *in situ* and mid-IR spectroscopy, it can be concluded that significant variation exists in structural and chemical composition among barley varieties. In addition, it is possible to select specific barley varieties for different purposes depending on these biochemical differences. Suitable barley grains for feed purpose are expected to have large particle size after simple processing and more starch and dietary protein can reach the small intestine to benefit the animal. Based on the *in situ* study, CDC Helgason exhibited the lowest degradation rate and extent of all nutrients (DM, CP and starch) among the six barley varieties, indicating that CDC Helgason may be the most ideal barley variety for feed purpose compared with the other five barley varieties. AC Metcalfe, a barley variety for malting purpose, has a faster degradation rate and greater degradation extent than feed type barely. An interesting result from this study is that CDC Trey, a barley variety for feed purpose, exhibits quite similar parameters in terms of particle size, degradation rate to the malting-purpose barley AC Metcalfe.

Inherent structural differences among the seeds of the six barley varieties in terms of hull, endosperm and whole seeds were detected by the DRIFT Spectroscopy and SFTIRM with univariate and multivariate spectral analyses. The results showed that the spectral profiling differed among the barley samples. The differences in particle size and structural and chemical make-up features may explain the various degradation characteristics exhibited in the rumen among the six barley varieties. Through application of these two multivariate techniques with the FTIR spectroscopy, it is possible to discriminate and classify the inherent molecular structural features among the different varieties. The AHCA and PCA enable us to deal with the informative data set. CDC Helgason was distinguished from AC Metcalfe, CDC Dolly, McLeod and CDC Cowboy in fingerprint (1800-800 cm^{-1}) and CHO region(1185-800 cm^{-1}), from AC Metcalfe, McLeod and CDC Cowboy in protein region (1715-1485 cm^{-1}). However, CDC Helgason cannot be distinguished from CDC Trey in either protein/CHO region or fingerprint region using these two methods with spectroscopic information though they have significant different degradation features. This suggests further spectroscopic analysis, such as IR imaging, FSD and 2nd derivative data treatment may be necessary. Mapping can also be utilized to identify the chemical compounds' distribution of region and track the difference. Besides the protein, CHO and fingerprint region, other spectral regions associated with nutrients (for example, CH component bands centered at 2925 cm^{-1} and CHO bands centered at 930 cm^{-1} and 860 cm^{-1}) can also be examined in the future.

Significant correlations were found between median particle size (not mean particle size) and the rate and extent of rumen degradation, but no strong correlations were found between spectral functional groups' intensity and their ratios and *in situ* data.

The studies demonstrated the potential of ultraspatially resolved synchrotron based technology (SFTIRM) to reveal the structural and chemical make-up within cellular and subcellular dimensions without destruction of the inherent structure of cereal seed tissue. With the SFTIRM techniques, the structural characteristics of the cereal seeds were illuminated among different cultivars at highly spatial resolution. The structural differences of barley seeds may be one reason for the various digestive behaviours and nutritive values for ruminants. Examination of other tissues such as aleurone layer and pericarp may provide further insight as to why barley verities exhibit different biodegradation behaviours, and provide a deeper and better indication of the chemical make-up and structural information.

The potential of mid-IR IR techniques in feed science has emerged from this study and other recent reports. More research is needed to achieve a deeper understanding of grains' complexity and improve these spectroscopic techniques to be more mature in feed science.

Industry Application

Infrared spectroscopy can be used in industrial and related field for quantitative and qualitative analysis of organic or inorganic ingredients. The rising number of research publications involving FTIR techniques in feed science heralds a broader future for commercial application. Such a tool can enable plant breeders to optimize cultivar selection strategies by rapidly analyzing the grains without chemical degradation. The genetic relationship of plants can be characterized and identified according to the spectral information. Combined with the multivariate spectral analyses (for example, AHCA and PCA), the FTIR techniques make it possible to characterise varieties in a short time. It can also be used for grain evaluation. The main constituents of grains are water, carbohydrates, protein, fat, fibre, and minerals. Using FTIR techniques, the structural and chemical composition information associated with nutrients can be rapidly obtained from a small volume of samples and make it possible to predict the feed quality to diminish the risk of metabolic disorders.

It is worth doing more studies to establish a rapid, non-destructive analytical technique to meet analytical needs. It can be developed as an effective method for nutritionists, cereal breeders, food and feed chemists, grain processors and scientists. To serve the industrial analytical requirement, there is a need to select representative samples for model establishment. A large number of analyses are also needed to ensure the accuracy. However, with a mature prediction models, feed samples can be evaluated directly and rapidly with minimal pre-treatment to obtain detailed information on chemical compounds.

Future Research

The application of FTIR techniques in combination with other chemical analysis can avoid structural information losses and evaluate the quality of barley grain more exactly and accurately. More study is needed to improve the effectiveness of analysis methods and provide further understanding of the relationship between structural and nutritional characteristics of feedstuffs. Suggestions for future research include expanded research to determine the relationship between feed inherent structure and degradation behaviours by using many different feedstuffs with a large range in rumen degradability. Further analysis is needed to determine differences in protein and carbohydrate molecular conformation among feedstuffs. Additionally, grain hardness, small and large starch granule ratio and conformation in endosperm tissue are also of interest. Partial Least Squares (PLS) analysis associated with Mid-IR spectroscopy can also be employed to provide deeper understanding of the structural and chemical make-up information of the feedstuffs.

Literature Cited (Part II and Thesis Two)

Association of Official Analytical Chemists. 1997. Official Methods of Analysis of the Association of Official Analytical Chemists, 16th ed. AOAC, Arlington, VA.

Bamba, T. 2002. In-situ chemical analyses of trans-polyisoprene by histochemical staining and Fourier transform infrared microspectroscopy in a rubber-producing plant, Eucommia ulmoides Oliver. Planta. 215 (6): 934.

Barth, A. 2007. Infrared spectroscopy of proteins. Biochim. Biophys. Acta. 1767 (9): 1073-1101.

Black, M. 2000. Seed technology and its biological basis. CRC Press, Boca Raton, Fla. 419 pp.

Bowman, J. G. P., Blake, T. K., Surber, L. M. M., Habernicht, D. K. and Bockelman, H. 2001. Feed-quality variation in the barley core collection of the USDA National Small Grains Collection. Crop Sci. 41 (3): 863-870.

Budevska, B. O. 2002. Vibrational spectroscopy imaging of agricultural products. Pages 3720-3732 *in* J. M. Chalmers, P. R. Griffiths, eds. Handbook of Vibrational Spectroscopy. J. Wiley, Hoboken, NJ.

Camm, G. A. 2008. Grain Hardness and Slow Dry Matter Disappearance Rate in Barley. M. Sc. thesis. University of Saskatchewan, Saskatoon, SK.

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. 2nd ed. Canadian Council on Animal Care, Ottawa. 298 pp.

Canadian Food Inspection Agency. 2007. List of varieties which are registered in Canada. [Online] Available: http://www.inspection.gc.ca/english/plaveg/variet/lovric.pdf [July, 2009].

Church, D. C. 1991. Livestock feeds and feeding. 3rd ed. Prentice Hall, Englewood Cliffs, NJ. 546 pp.

Davies, T. and Fearn, T. 2004. Back to basics: the principles of principal component analysis. Spectrosc. Eur. 16 (6): 20.

Diem, M., Romeo, M., Matthäs, C., Miljkovic, M., Miller, L. and Lasch, P. 2004. Comparison of Fourier transform infrared (FTIR) spectra of individual cells acquired using synchrotron and conventional sources. Infrared Phys. Technol. 45 (5-6): 331-338.

Doiron, K. 2009. Characterization of autoclaved flaxseed as feed for ruminants using conventional and Mid-IR spectroscopic based approaches. M. Sc. thesis. University of Saskatchewan, Saskatoon, SK.

Du, L. 2009. Hull, ferulic acid, para-coumaric acid content and particle size characteristics of various barley varieties in relation to nutrient availability in ruminants. M. Sc. thesis. University of Saskatchewan, Saskatoon, SK.

Du, L., Yu, P., Rossnagel, B. G., Christensen, D. A. and McKinnon, J. J. 2009. Physicochemical characteristics, hydroxycinnamic acids (ferulic acid, P-coumaric acid) and their ratio, and *in situ* biodegradability: comparison of genotypic differences among six barley varieties. J. Agric. Food Chem. 57 (11): 4777-4783.

Dumas, P., Jamin, N., Teillaud, J. L., Miller, L. M. and Beccard, B. 2004. Imaging capabilities of synchrotron infrared microspectroscopy. Faraday Discuss. 126: 289-302.

Dumas, P. and Miller, L. 2003. The use of synchrotron infrared microspectroscopy in biological and biomedical investigations. Vib. Spectrosc. 32 (1): 3-21.

Dumas, P., Sockalingum, G. D. and Sule-Suso, J. 2007. Adding synchrotron radiation to infrared microspectroscopy: what's new in biomedical applications? Trends Biotechnol. 25 (1): 40-44.

Dunteman, G. H. 1989. Principal components analysis. Sage Publications, Newbury Park, CA. 96 pp.

Figroid, W., Hale, W. H. and Theurer, B. 1972. An evaluation of the nylon bag technique for estimating rumen of grains. J. Anim. Sci. 35 (1): 113-120.

Foley, A. E., Hristov, A. N., Melgar, A., Ropp, J. K., Etter, R. P., Zaman, S., Hunt, C. W., Huber, K. and Price, W. J. 2006. Effect of barley and its amylopectin content on ruminal fermentation and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89 (11): 4321-4335.

Food and Agriculture Organization. 2007. Food balance sheet-excluding beer. [Online] Available: http://faostat.fao.org/default.aspx [July, 2009].

Gan, G., Ma, C. and Wu, J. 2007. Data Clustering: Theory, Algorithms, and Applications (ASA-SIAM Series on Statistics and Applied Probability). Society for Industrial and Applied Mathematics Philadelphia . PA. 466 pp.

Griffiths, P. R. and De Haseth, J. A. 1986. Fourier transform infrared spectrometry. Wiley, New York. 656 pp.

Hale, W. H. 1973. Influence of processing on the utilization of grains (starch) by ruminants. J. Anim. Sci. 37 (4): 1075.

Hart, K. J., Rossnagel, B. G. and Yu, P. 2008. Chemical characteristics and in situ ruminal parameters of barley for cattle: Comparison of the malting cultivar AC Metcalfe and five feed cultivars. Can. J. Anim. Sci. 88: 711-719.

Harvey, B. L. and Rossnagel, B. G. 1984. Harrington barley. Can. J. Plant Sci. 64(1): 193-194.

Herrera-Saldana, R. E., Huber, J. T. and Poore, M. H. 1990. Dry matter, crude protein, and starch degradability of five cereal grains. J. Dairy Sci. 73 (9): 2386-2393.

Himmelsbach, D. S., Khalili, S. and Akin, D. E. 1998. FT-IR microspectroscopic imaging of flax (Linum usitatissimum L.) stems. Cell. Mol. Biol. (Noisy-Le-Grand). 44 (1): 99-108.

Hindle, V. A., Vuuren van, A. M., Klop, A., Mathijssen-Kamman, A. A., van Gelder, A. H. and Cone, J. W. 2005. Site and extent of starch degradation in the dairy cow - a comparison between *in vivo*, *in situ* and *in vitro* measurements. J. Anim. Physiol. Anim. Nutr. 89 (3-6): 158-165.

Holopainen, U. R., Wilhelmson, A., Salmenkallio-Marttila, M., Peltonen-Sainio, P., Rajala, A., Reinikainen, P., Kotaviita, E., Simolin, H. and Home, S. 2005. Endosperm structure affects the malting quality of barley (*Hordeum vulgare* L.). J. Agric. Food Chem. 53 (18): 7279-7287.

Holtekjøen, A. K., Uhlen, A. K., Bråhen, E., Sahlstrø, S. and Knutsen, S. H. 2006. Contents of starch and non-starch polysaccharides in barley varieties of different origin. Food Chem. 94 (3): 348-358.

Homan, J. A., Radel, J. D., Wallace, D. D., Wetzel, D. L. and Levine, S. M. 2000. Chemical changes in the photoreceptor outer segments due to iron induced oxidative stress: analysis by Fourier transform infrared (FT-IR) microspectroscopy. Cell. Mol. Biol. (Noisy-Le-Grand). 46 (3): 663-672.

Jackson, M. and Mantsch, H. H. 1991. Protein secondary structure from FT-IR spectroscopy: Correlation with dihedral angles from three-dimensional Ramachandran plots. Can. J. Chem. 69 (11): 1639-1642.

Jackson, M. and Mantsch, H. H. 2000. Infrared spectroscopy ex vivo tissue analysis. Pages 131-156 *in* RA Myers, ed. Encyclopedia of Analytical Chemistry. John Wiley & Sons Ltd, Chichester, England.

Jain, A. K., Murty, M. N. and Flynn, P. J. 1999. Data clustering: a review. ACM computing surveys. 31 (3): 264 - 323.

Jolliffe, I. T. 2002. Principal component analysis. 2nd ed. Springer, New York, NY, 487pp.

Kaufman, L. and Rousseeuw, P. J. 1990. Finding groups in data: an introduction to cluster analysis. Wiley, New York. 342 pp.

Keunen, J. E., Plaizier, J. C., Kyriazakis, L., Duffield, T. F., Widowski, T. M., Lindinger, M. I. and McBride, B. W. 2002. Effects of a subacute ruminal acidosis model on the diet selection of dairy cows. J. Dairy Sci. 85 (12): 3304-3313.

Kellems, R. O. and Church, D. C. 2002. Livestock feeds and feeding. 5th ed. Prentice Hall, Upper Saddle River, NJ. 654 pp.

Khorasani, G. R., Helm, J. and Kennelly, J. J. 2000. *In situ* rumen degradation characteristics of sixty cultivars of barley grain. Can. J. Anim. Sci. 80 (4): 691-701.

Kulp, K. and Ponte, J. G. 2000. Handbook of cereal science and technology. 2nd , rev. and expand ed. Marcel Dekker, New York. 790 pp.

Langrell, D. E., Edney, M. J. and Izydorczyk, M. S. 2008. Quality of western Canadian malting barley. Canadian Grain Commission, Winnipeg.

Legge, W. G. 2003. AC Metcalfe barley. Can. J. Plant Sci. 83 (2): 381-384.

LeVine, S. M., Radel, J. D., Sweat, J. A. and Wetzel, D. L. 1999. Microchemical analysis of retina layers in pigmented and albino rats by Fourier transform infrared microspectroscopy. Biochim. Biophys. Acta. 1473 (2-3): 409-417.

LeVine, S. M. and Wetzel, D. L. 1994. *In situ* chemical analyses from frozen tissue sections by Fourier transform infrared microspectroscopy. Examination of white matter exposed to extravasated blood in the rat brain. Am. J. Pathol. 145 (5): 1041-1047.

LeVine, S. M. and Wetzel, D. L. 1998. Chemical analysis of multiple sclerosis lesions by FT-IR microspectroscopy. Free Radic. Biol. Med. 25 (1): 33-41.

Levine, S. M. and Wetzel, D. L. B. 1993. Analysis of brain tissue by FT-IR microspectroscopy. Appl. Spectrosc. Rev. 28 (4): 385-412.

Marinkovic, N. S. and Chance, M. R. 2005. Synchrotron infrared microspectroscopy. Page 671–708 *in* R. Meyers, ed. Encyclopedia of Molecular Cell Biology and Molecular Medicine. Wiley, New York.

Marinkovic, N. S., Huang, R., Bromberg, P., Sullivan, M., Sperber, E., Moshe, S., Miller, L. M., Jones, K., Chouparova, E. and Franzen, S. 2002. Center for Synchrotron Biosciences' U2B Beamline: an international resource for biological infrared spectroscopy. J. Synchrotron Rad. 9 (part 4): 189–197.

Messerschmidt, R. G. and Harthcock, M. A. 1988. Infrared microspectroscopy: theory and applications. H. Dekker, New York, NY. 282 pp.

Mikel, M. A. and Kolb, F. L. 2008. Genetic Diversity of Contemporary North American Barley. Crop Sci. 48 (4): 1399-1407.

Miller, L. M. 2002. Infrared microspectroscopy and imaging. National Synchrotron Light Source, Brookhaven National Laboratory, Internal publication. [Online] Available: http://www.nsls.bnl.gov/newsroom/publications/otherpubs/imaging/workshopmiller.pdf [July, 2009].

Miller, L. M., Carr, G. L., Jackson, M., Dumas, P. and Williams, G. P. 2000. The impact of infrared synchrotron radiation in biology: Past, present and future. Synchrotron Radiation News. 13 (5): 31-38.

Miller, L. M. and Dumas, P. 2006. Chemical imaging of biological tissue with synchrotron infrared light. Biochim. Biophys. Acta. 1758 (7): 846-857.

Miller, L. M., Wang, Q., Smith, R. J., Zhong, H., Elliott, D. and Warren, J. 2007. A new sample substrate for imaging and correlating organic and trace metal composition in biological cells and tissues. Anal. Bioanal. Chem. 387 (5): 1705-1715.

Mills, E. N. C., Parker, M. L., Wellner, N., Toole, G., Feeney, K. and Shewry, P. R. 2005. Chemical imaging: the distribution of ions and molecules in developing and mature wheat grain. J. Cereal Sci. 41 (2): 193-201.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. National Academy Press, Washington, D.C.

Nelson, D. L. and Cox, M. M. 2005. Lehninger principles of biochemistry. WH Freeman, New York, NY 1119 pp.

Nocek, J. E. 1988. *In situ* and other methods to estimate ruminal protein and energy digestibility: a review. J. Dairy Sci. 71 (8): 2051-2069.

Nocek, J. E. and Tamminga, S. 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74 (10): 3598-3629.

OMAF Feed Advisory Program. 2009. Cereal Crops - Barley, Oats, Wheat. [Online] Available:

http://www.omafra.gov.on.ca/english/crops/field/cereal.html [July, 2009].

Ørskov, E. R. 1986. Starch digestion and utilization in ruminants. J. Anim. Sci. 63 (5): 1624-1633.

Ørskov, E. R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92 (part 2): 499-503.

Pietrzak, L. N. and Miller, S. S. 2005. Microchemical structure of soybean seeds revealed *in situ* by ultraspatially resolved synchrotron Fourier transformed infrared microspectroscopy. J. Agric. Food Chem. 53 (24): 9304-9311.

Plaizier, J. C., Krause, D. O., Gozho, G. N. and McBride, B. W. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet. J. 176 (1): 21-31.

Raab, T. K. and Martin, M. C. 2001. Visualizing rhizosphere chemistry of legumes with mid-infrared synchrotron radiation. Planta. 213 (6): 881-887.

Romesburg, H. C. 1984. Cluster analysis for researchers. Lifetime Learning Publications, Belmont, CA. 334 pp.

Rossnagel, B. G. and Harvey, B. L. 1994. CDC Dolly Two-row Feed Barley. 38: 109. Barley Newsletter. American Malting Barley Association, Inc., Milwaukee, WI.

SAS. 2003. User's guide: Statistics. 9th ed. SAS Institute, Inc., Cary, NC.

Slafer, G. A. 2002. Barley science: recent advances from molecular biology to agronomy of yield and quality. Food Products Press, Binghamton, NY. 565 pp.

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G. and Russell, J. B. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70 (11): 3562-3577.

Stuart, B. 2004. Infrared spectroscopy: fundamentals and applications. J. Wiley, Chichester, West Sussex, England; Hoboken, NJ.

Takeda, Y., Takeda, C., Mizukami, H. and Hanashiro, I. 1999. Structures of large, medium and small starch granules of barley grain. Carbohydr. Polym. 38 (2): 109-114.

Tamminga, S., Van Straalen, W. M., Subnel, A. P. J., Meijer, R. G. M., Steg, A., Wever, C. J. G. and Blok, M. C. 1994. The Dutch protein evaluation system: the DVE/OEB-system. Livest. Prod. Sci. 40 (2): 139-155.

Tamminga, S., Vuuren, A. M., Koelen, C. J., Ketelaar, R. S. and Togt, P. L. 1990. Ruminal behaviour of structural carbohydrates, non-structural carbohydrates and crude protein from concentrate ingredients in dairy cows. Neth. J. Agric. Sci. 38 (3): 513-526.

Tester, R. F., Karkalas, J. and Qi, X. 2004. Starch—composition, fine structure and architecture. J. Cereal Sci. 39 (2): 151-165.

Theurer, C. B. 1986. Grain processing effects on starch utilization by ruminants. J. Anim. Sci. 63 (5): 1649-1662.

Valentin, S. F., Williams, P. E. V., Forbes, J. M. and Sauvant, D. 1999. Comparison of the in vitro gas production technique and the nylon bag degradability technique to measure short-and long-term processes of degradation of maize silage in dairy cows. Anim. Feed Sci. Technol. 78 (1-2): 81-99.

von Bothmer, R. 2003. Diversity in barley: (*Hordeum vulgare*). 1st ed. Elsevier, Amsterdam; New York. 280 pp.

Waldo, D. R. 1973. Extent and partition of cereal grain starch digestion in ruminants. J. Anim. Sci. 37 (4):

1062-1074.

Welch, J. G. 1986. Physical parameters of fiber affecting passage from the rumen. J. Dairy Sci. 69 (10): 2750.

Wetzel, D. L. 1993. Molecular Mapping of Grain with a Dedicated Integrated Fourier Transform Infrared Microspectrometer. Dev. Food Sci. 32: 679-679.

Wetzel, D. L. 1998. Metabolically deuterated species determined in rat cerebella by FT-IR microspectroscopy as a novel probe of brain metabolism. Pages 294 AIP conference proceedings. American Institute of Physics, New York.

Wetzel, D. L. 2000. Infrared spectroscopy goes microscopic. Chem. Ind. (London). 9 (8): 308-313.

Wetzel, D. L., Eilert, A. J., Pietrzak, L. N., Miller, S. S. and Sweat, J. A. 1998a. Ultraspatially-resolved synchrotron infrared microspectroscopy of plant tissue *in situ*. Cell. Mol. Biol. (Noisy-Le-Grand). 44 (1): 145-168.

Wetzel, D. L. and LeVine, S. M. 1993. *In situ* FT-IR microspectroscopy and mapping of normal brain tissue. Spectroscopy. 8: 40-45.

Wetzel, D. L. and LeVine, S. M. 2000. Infrared microbeam analysis of intricate biological specimens. Inst. Phys. Conf. Ser. 165: 65-66.

Wetzel, D. L. and LeVine, S. M. 2001. Biological applications of infrared microspectroscopy. Pages 101-142 *in* Hans-Ulrich Gremlich and Bing Yan, ed. Infrared and Raman Spectroscopy of Biological Materials. Marcel Dekker Inc., NY.

Wetzel, D. L., Reffner, J. A., Carr, G. L. and Cho, L. 1998b. Synchrotron powered FT-IR microspectroscopy permits small spot ATR sampling of fiber finish and other materials. Pages 657-660 *in* J. A. d. Haseth, ed. Fourier Transform Spectroscopy. American Institute of Physics, Woodbury, NY.

Wetzel, D. L., Slatkin, D. N. and Levine, S. M. 1998c. FT-IR microspectroscopic detection of metabolically deuterated compounds in the rat cerebellum: a novel approach for the study of brain metabolism. Cell. Mol. Biol. (Noisy-Le-Grand). 44 (1): 15-27.

Yang, W. Z., Beauchemin, K. A., Farr, B. I. and Rode, L. M. 1997. Comparison of barley, hull-less barley, and corn in the concentrate of dairy cows. J. Dairy Sci. 80 (11): 2885-2895.

Yoshimoto, Y., Tashiro, J., Takenouchi, T. and Takeda, Y. 2000. Molecular structure and some physicochemical properties of high-amylose barley starches. Cereal Chem. 77 (3): 279-285.

You, S. G. and Izydorczyk, M. S. 2002. Molecular characteristics of barley starches with variable amylose content. Carbohydr. Polym. 49 (1): 33-42.

Yu, P. 2004. Application of advanced synchrotron radiation-based Fourier transform infrared (SR-FTIR) microspectroscopy to animal nutrition and feed science: a novel approach. Br. J. Nutr. 92 (6): 869-885.

Yu, P. 2005a. Application of cluster analysis (CLA) in feed chemical imaging to accurately reveal structural-chemical features of feeds and plants within cellular dimension. J. Agric. Food Chem. 53 (8): 2872-2880.

Yu, P. 2005b. Applications of hierarchical cluster analysis (CLA) and principal component analysis (PCA) in feed structure and feed molecular chemistry research, using synchrotron-based Fourier transform infrared (FTIR) microspectroscopy. J. Agric. Food Chem. 53 (18): 7115-7127.

Yu, P. 2005c. Multicomponent peak modeling of protein secondary structures: comparison of gaussian with lorentzian analytical methods for plant feed and seed molecular biology and chemistry research. Appl. Spectrosc. 59 (11): 1372-1380.

Yu, P. 2006a. An emerging method for rapid characterization of feed structures and feed component matrix at a cellular level and relation to feed quality and nutritive value. Arch. Anim. Nutr. 60 (3): 229-244.

Yu, P. 2006b. Synchrotron IR microspectroscopy for protein structure analysis: Potential and questions. Spectroscopy. 20 (5): 229-251.

Yu, P. 2007a. Molecular chemical structure of barley proteins revealed by ultra-spatially resolved synchrotron light sourced FTIR microspectroscopy: comparison of barley varieties. Biopolymers. 85 (4): 308-317.

Yu, P. 2007b. Protein secondary structures (α-helix and β-sheet) at a cellular level and protein fractions in relation to rumen degradation behaviours of protein: a new approach. Br. J. Nutr. 94 (05): 655-665.

Yu, P. 2007c. Ultra-spatial synchrotron radiation for imaging molecular chemical structure: Applications in plant and animal studies. Spectroscopy. 21 (4): 183-192.

Yu, P. 2008. Modeling protein structures in feed and seed tissues using novel synchrotron-based analytical technique. Anim. Feed Sci. Technol. 140 (1-2): 199-206.

Yu, P., Block, H., Niu, Z. and Doiron, K. 2007. Rapid characterization of molecular chemistry, nutrient make-up and microlocation of internal seed tissue. J Synchrotron Rad. 14 (part 4): 382-390.

Yu, P., Block, H. C. and Doiron, K. 2009a. Understanding the differences in molecular conformation of carbohydrate and protein in endosperm tissues of grains with different biodegradation kinetics using advanced synchrotron technology. Spectroc. Acta Pt.A-Molec. Biomolec. Spectr. 71 (5): 1837-1844.

Yu, P., Christensen, C. R., Christensen, D. A. and McKinnon, J. J. 2005. Ultrastructural-chemical make-up of yellow-seeded (Brassica rapa) and brown-seeded (Brassica napus) canola within cellular dimensions, explored with synchrotron reflection FTIR microspectroscopy. Can. J. Plant Sci. 85 (3): 533-542.

Yu, P., Christensen, D. A., Christensen, C. R., Drew, M. D., Rossnagel, B. G. and McKinnon, J. J. 2004a. Use of synchrotron FTIR microspectroscopy to identify chemical differences in barley endosperm tissue in relation to rumen degradation characteristics. Can. J. Anim. Sci. 84 (3): 523-528.

Yu, P., Doiron, K. and Liu, D. 2008. Shining light on the differences in molecular structural chemical make-up and the cause of distinct degradation behavior between malting- and feed-type barley using synchrotron FTIR microspectroscopy: a novel approach. J. Agric. Food Chem. 56 (9): 3417-3426.

Yu, P., Jonker, A. and Gruber, M. 2009b. Molecular basis of protein structure in proanthocyanidin and anthocyanin-enhanced Lc-transgenic alfalfa in relation to nutritive value using synchrotron-radiation FTIR microspectroscopy: A novel approach. Spectroc. Acta Pt.A-Molec. Biomolec. Spectr. 73 (5): 846-853.

Yu, P., McKinnon, J. J., Christensen, C. R. and Christensen, D. A. 2004b. Imaging molecular chemistry of Pioneer corn. J. Agric. Food Chem. 52 (24): 7345-7352.

Yu, P., McKinnon, J. J., Christensen, C. R. and Christensen, D. A. 2004c. Using synchrotron-based FTIR microspectroscopy to reveal chemical features of feather protein secondary structure: comparison with other feed protein sources. J. Agric. Food Chem. 52 (24): 7353-7361.

Yu, P., McKinnon, J. J., Christensen, C. R. and Christensen, D. A. 2004d. Using Synchrotron Transmission FTIR Microspectroscopy as a Rapid, Direct, and Nondestructive Analytical Technique To Reveal Molecular Microstructural- Chemical Features within Tissue in Grain Barley. J. Agric. Food Chem. 52 (6): 1484-1494.

Yu, P., Meier, J. A., Christensen, D. A., Rossnagel, B. G. and McKinnon, J. J. 2003. Using the NRC-2001 model and the DVE/OEB system to evaluate nutritive values of Harrington(malting-type) and Valier(feed-type) barley for ruminants. Anim. Feed Sci. Technol. 107 (1-4): 45-60.

Appendices

PART I:

Appendix 1.

Comparison of Modified EBC Method and 50% H_2SO_4 Method (Whitmore 1961) in Determinantion of Barley Hull Content

A1.1 Modified EBC Method for Barley Hull Content Analysis

Four randomly selected barley samples were used for EBC method testing. They were AC Metcalfe (2003), RCSL 97 (2003), AC Metcalfe (2004) and CDC Trey (2004). Barley grain samples were screened according to EBC method (EBC 3.9 1998). Naked and broken seeds were removed by hand. 20 g of barley grain was boiled and digested for 3 min in the solution of 80 ml sodium hypochlorite (12%) and 20 ml sodium hydroxide (3.125 N). The dehulled samples were then dried and ground to determine barley hull content.

A1.2 50% H_2SO_4 Method (Whitmore 1961) for Barley Hull Content Analysis

The dehulling procedure of 50% H_2SO_4 (7664-93-9, 98%, VWR International, Pennsylvania, USA) (Whitmore 1961) was similar to the modified EBC method, except that 20 g samples were immersed in 100 ml 50% H_2SO_4 at room temperature for 3 h to remove the hull. The dehulled samples were also dried and ground to determine barley hull content.

A1.3 Comparison between Modified EBC Method and 50% H_2SO_4 Method for Barley Hull Content Analysis

Barley grain contains a protective outer hull, which consists of lemma (attached to the dorsal side) and palea (attached to the ventral side) (Hough 1991; Dendy and Dobraszczyk 2001). The hull is thin compared to that in many other grains such as oat and rice, but it plays an important role by providing physical protection for the grain, supporting the growing acrospires, maintaining water balance, and even plays a role in photosynthesis when the hull is green (Olkku et al. 2005). In hull-less barley, the hull loosely sticks to the caryopsis and sheds easily during harvesting and threshing, while in hulled barley, it is more tightly adhered to the pericarp, which is removable, but with difficulty (Evers et al. 1999). In barley, the hull is cemented to the pericarp and it is difficult to distinguish between the two by visual observation. This also makes mechanical and/or chemical dehulling difficult. So far, there is no accurate method for completely separating the hull from barley grain without compromising the pericarp or endosperm. Most of the existing methods such as the EBC method (EBC 1998) are suitable for comparison analysis. However, the high concentration of sodium hypochlorite solution (20% NaClO) required in the EBC procedure was not available for our experiment. Therefore, a relatively low concentration (12% NaClO) was used and the reacting time was adjusted from 80 sec to 3 min. The modified EBC method was compared with a method using 50% H_2SO_4 (Whitmore 1961).

Results from the modified EBC method and the 50% H_2SO_4 method are presented in Table A1.1. The hull content was slightly higher with the modified EBC method than with 50% H_2SO_4. However, both methods gave a similar trend in hull content and were highly correlated (R = 0.91). These results indicate that the modified EBC method is an acceptable method to compare differences in barley hull content among various barley varieties. The 50% H_2SO_4 method was difficult and dangerous to handle and quiet tedious, therefore, the modified EBC method was employed for analyzing barley hull content in the present study.

Table A1. Comparison of two methods for determination of barley hull content

Barley sample	Hull content (%DM)	
	Modified EBC method	50% H_2SO_4 method
AC Metcalfe, 2003	10.8 ± 0.1	9.2 ± 0.3
RCSL 97, 2003	10.3 ± 0.2	9.1 ± 0.2
CDC Trey, 2004	10.1 ± 0.1	8.2 ± 0.3
AC Metcalfe, 2004	9.1 ± 0.03	7.8 ± 0.1
Correlation coefficient (R)	0.91	

Appendix 2. Pretreatment before Alkaline Hydrolysis for Barley Grain

Barley grain contains a high content of starch which produces problems by swelling and gelation during alkaline hydrolysis and extraction and even during HPLC analysis. Researchers have introduced large quantities of barley grain (400-1600 mg) and washed the starch enriched grain powders with large volumes of ethanol and hexane (100 ml/g) (Nordkvist et al. 1984; Andreasen et al. 2000; Holtekjolen et al. 2006) to reduce the swelling and gelation problems. The washing step could eliminate some of the starch problems, but could still not work well in the present study. During saponification, the treated samples would swell to form a thick gelation which would complicate the hydrolysis and extraction. Therefore, we developed a new method to overcome the problem. We had tried several tests to optimize the reaction conditions, including incubation in hot water with heating, incubation in α-Amylase solution with cool water and hydrolyzing for different time (20 min, 30 min or 1 h), and incubation in α-Amylase solution in 90 °C for 20 min, 30 min or 1 h. After comparison, we found the best way to reduce swelling and gelating problem was to mix the ground barley grain (50 mg) with 0.75 ml 1% (w/v) α-Amylase dissolved in 20mM KH_2PO_4 buffer (pH 6.9), and incubated in 90 °C water for 1 h.

Appendix 3. HPLC chromatogram of PCA and FA in barley grain

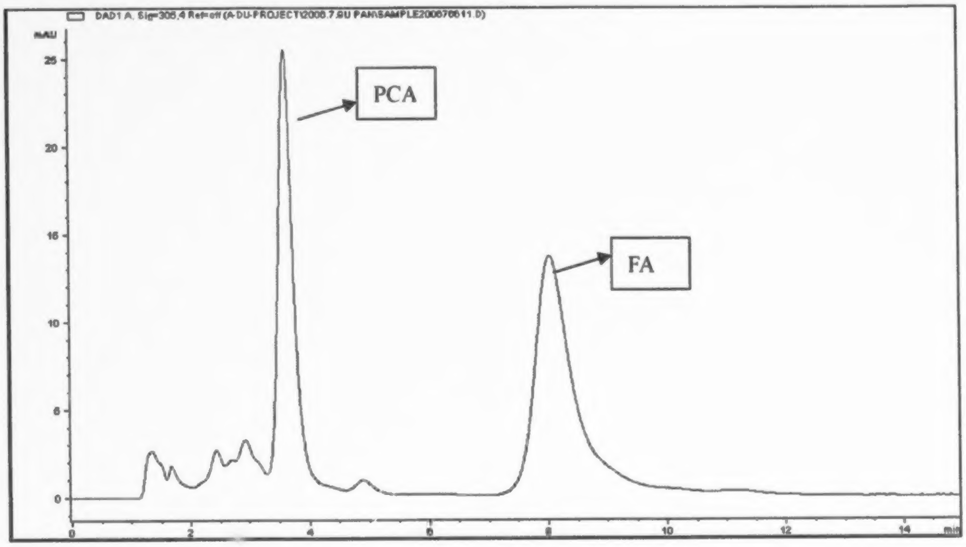


Figure A1. HPLC chromatogram at 305 nm of PCA and FA in barley grain extracted after alkaline hydrolysis

Appendix 4. Selection of the Best Model for Determining Mean/Median Particle Size of Coarsely Dry-rolled Barley Grain

A4.1 Mean/Median Particle Size Calculation with Three Equations

After sieving, the fractions remaining on each screen were weighed, and particle size distribution was expressed in percentage cumulative weight oversize by adding up the weight on each sieve and those from all larger screens (ANSI 2003). The mean/median particle size values were estimated by fitting these data into two exponential models: Fisher's equation (A1) (Fisher et al. 1988) and Pond's equation (A2) (Pond et al. 1984) with/without 0 mm = 100%. The particles passing the sieve 0.58 mm were included in Pond's equation with 0 mm = 100%, and discarded in Pond's equation without 0 mm = 100% in the calculation. Data were computed using the NLIN procedure of the Statistical Analytical System (SAS) (SAS Institute, Inc. 2002). Mean particle size was calculated as the weighted average of sample particle sizes and median particle size was determined to be equivalent to the value at 50% of the percentage cumulative weight oversize.

Pond's Equation

$$R = 100\, e^{-k(s-w)} \qquad \text{(A1)}$$

mean particle size = $1/k+w$

median particle size = 0.693/k+w.

Fisher's Equation

$$R = 100e^{-(S^{a}-b \cdot s)} \qquad \text{(A2)}$$

Where:

R = percentage cumulative weight oversize;
s = sieve opening size (mm);
w = the smallest predictable particle size;
k = the decay constant of the exponential curve describes the proportionality constant between the percent of particles passed to the next sieve and the percent remained.
a and b = mathematically estimated parameters.

Although the particle size of coarsely dry-rolled barley samples was not logarithmic-normally distributed, geometric mean diameter (GM) (A3) (ANSI 2003) was still calculated for the sake of equation comparison. The average particle size of materials retained on each sieve was fitted into the log-normal distribution curve (ANSI 2003) and the geometric mean diameter was calculated accordingly.

Equation for Predicting Geometric Mean Diameter (GM) of Log-normal Distribution

$$d_{gw} = \log^{-1}\left[\frac{\sum_{i=1}^{n} (W_i \log \bar{d}_i)}{\sum_{i=1}^{n} W_i}\right] \qquad \text{(A3)}$$

where:

d_{gw} = geometric mean or median particle size of the whole materials, mm
d_i = nominal sieve opening of the ith screen, mm
W_i = the weight of particles retained on ith sieve, g

A4.2 Selection of the Best Model

Particle size distribution data (percentage cumulative weight oversize) was analyzed with three equations using the Gauss-Newton nonlinear iterative method. The statistical parameters used for equation comparison and selection of the best model were residue sum of squares (RSS) and coefficient of determination (R^2). Fisher et al (1988) only adopted RSS for their comparative study, while Pasikatan et al. (1999) used four indices to test eight distribution models for ground wheat. The best equation suitable for analyzing coarsely dry-rolled barley samples should give the smallest RSS value, with the largest R^2 value. In Table A2, the parameters of RSS and R^2, as well as the mean/median particle size predicted from Fisher's equation, Pond's equation (with and without 0 mm = 100%) and geometric mean diameter (GM) calculation equation were compared.

Table A2. Comparison of the parameters of RSS and R^2, as well as mean/median particle size predicted from Fisher's equation and Pond's equation (with/without 0 mm = 100%) for coarsely dry-rolled barley grain

Equations	Mean (mm)	Median (mm)	RSS	R^2
Fisher's	3.55^{a}	3.09^{a}	363.21^{a}	0.9917^{b}
Pond's (with 0 mm=100%)	3.35^{b}	2.91^{b}	68.66^{b}	0.9987^{a}
Pond's (without 0 mm=100%)	3.35^{b}	2.91^{b}	68.62^{b}	0.9984^{a}
Geometric Mean (GM)	2.75^{c}			
SEM	0.053	0.045	8.049	0.00049
P value			<0.05	

$^{a, b, c, d}$ Different superscripts of in the same column are significantly different ($P < 0.05$).

RSS from Pond's equation with and without 0 mm = 100% were 68.66 and 68.62, respectively, not significantly different ($P>0.05$). However, both values were significantly smaller ($P<0.05$) than RSS from Fisher's equation, indicating that Pond's equation was more suitable to model particle size data from coarsely dry-rolled barley grain than Fisher's equation. R^2 values ($P<0.001$) continued to support the point that Pond's equation (R^2: 0.9987, 0.9984) was better than Fisher's equation ($R^2 = 0.9917$). Within Pond's methods, no difference was found for RSS and R^2, but better potency was observed in Pond's equation with 0 mm = 100%, which included the observation of particles passing through the smallest sieve (0.58 mm). R^2 for Pond's equation with 0 mm = 100% was 0.9987. The estimation of mean/median particle sizes from Fisher's equation was larger than those from Pond's and GM calculation equation, with GM giving the smallest particle size.

Since the two parameters (RSS, R^2) denoted that Pond's equation was the best choice, in the following calculation and comparison, the Pond's equation with 0 mm = 100% was applied for computing mean/median particle size of coarsely dry-rolled barley samples expressed as percent cumulative weight oversize.

Appendix 5. Animal Diets during In Situ Experiments

Table A3. Standard dairy concentrate[1,2]

Integrant	%DM
Barley	56
Wheat	5
Oats	5
Dairy supplement pellets	33
Molasses	1

[1] Grain was dry rolled and mixed with supplement pellets.

[2] Proximate composition: 18.5% crude protein, 0.7% calcium, 0.8% phosphorus (DM basis).

Table A4. Fresh cow concentrate[1,2]

Integrant	%DM
Barley	51.05
Oats	5.0
Canola meal	11.6
Soybean meal	10.0
Wheat distillers dried grain	9.0
Corn gluten meal	3.0
Molasses	2.5
Golden flakes[3]	2.5
Canola oil	0.5
Mineral-vitamin mix[4]	3.0
Niacin-magnesium mix[5]	0.3
Cobalt-iodized salt	0.6
Sodium bicarbonate	0.6

Ground limestone	0.3
Dynamate[6]	0.05

[1] 0.48 cm (3/16") pellets

[2] Proximate composition: 22% crude protein, 0.9% calcium, 0.85% phosphorus (DM basis).

[3] Dried fat supplement (Malaysian palm oil) distributed in Western Canada by Prairie Micro-Tech Inc., Regina, Saskatchewan.

[4] Formulated to provide 45 mg manganese, 63 mg zinc, 17 mg copper, 0.5mg selenium, 11000 I.U. vitamin A, 1800 I.U. Vitamin E per kg of dairy concentrate. The mix also contributes 0.14% magnesium, 0.48% calcium, 0.26% phosphorus, 0.23% sodium and 0.38% chloride to the total dairy concentrate. Prepared by Federated Co. Ltd., Saskatoon, Saskatchewan.

[5] Formulated to provide 1 g of niacin and 0.3 g of magnesium per kg of fresh cow concentrate.

[6] Contains 22% sulphur, 18% potassium, 11% magnesium (International Minerals and Chemical Corp., Mundelein, ILL).

Appendix 6. Nylon Bags and Incubation Time Arrangements for In Situ Experiments

Table A5. Nylon bags arrangement for 0 - 72 h of rumen incubation

Incubation period (h)	Bags/ period	Treat- ments	Total bags	% of total #bags	Assumed #bags /mesh bag /animal	Assumed #bags Incubated /animal	Real #bags incubated /animal
0 h[1]	2	6	-	-	-	-	-
2 h	2	6	12	0.08	30	2.5	3
4 h	2	6	12	0.08	30	2.5	3
8 h	2	6	12	0.08	30	2.5	3
12 h	3	6	18	0.13	30	3.7	4
24 h	4	6	24	0.17	30	5.0	5
48 h	5	6	30	0.21	30	6.4	6
72 h	6	6	36	0.25	30	7.5	8
Total	24		144	1		30	32

[1] Bags were not counted for rumen incubation bags at 0 h.

Table A6. Detailed 'gradual in/all out' rumen incubation schedule

Day	Incubation time	Incubation period (h)	# bags incubated in cow 1	# bags incubated in cow 2
day 1	21:00	72	8	8
day 2	21:00	48	6	6
day 3	21:00	24	5	5
day 4	9:00	12	4	4
	13:00	8	3	3
	17:00	4	3	3
	19:00	2	3	3
	21:00		32[1]	32[1]

[1] Total bags incubated in each animal in a consecutive incubation.

Appendices

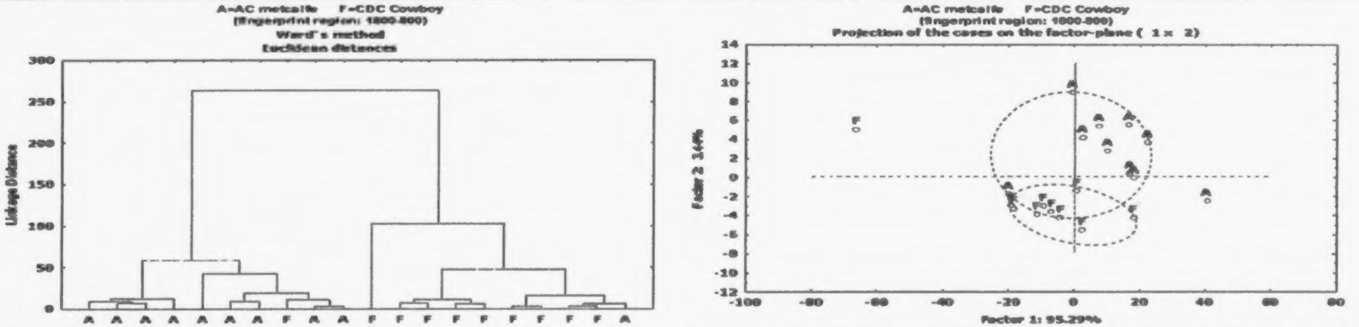
PART II:

APPENDIX: SUPPLEMENTARY MATERIAL

Appendix 1. Using Mid-IR spectroscopy with multivariate molecular spectral analyses to discriminate and classify molecular structure difference of barley seed: expanded comparison

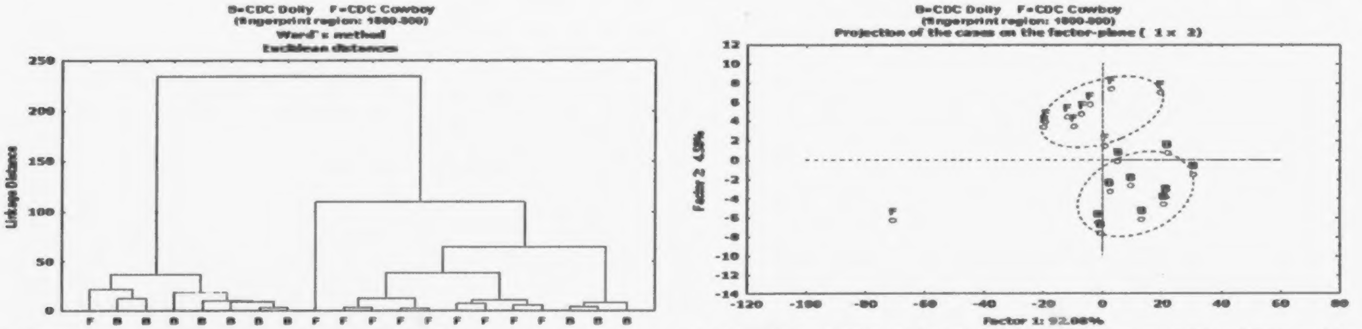
Paired comparisons were conducted among the six barley varieties using AHCA and PCA analysis. Besides the results shown in Chapter 3 using DRIFT spectroscopic data collected from whole seed sample to compare CDC Helgason with other five varieties in fingerprint, protein and CHO region, the results also indicated that CDC Cowboy differed with McLeod and CDC Trey in fingerprint region (Figure A1); differed with CDC Trey in protein region(Figure A2); differed with CDC Dolly, McLeod and CDC Trey in CHO region (Figure A3). DRIFT spectral data of hull sample from the six barley varieties and the SFTIR spectral data of endosperm tissue were also analyzed using these two multivariate methods. However, all the varieties comparison could not be grouped in separated cluster (for AHCA) or ellipse (for PCA) due to overlapping.

I: Cluster Analysis (CLA): fingerprint region 1800-800 cm^{-1} II: Principal component analysis (PCA): fingerprint region 1800-800 cm^{-1}



(1) Comparison of AC Metcalfe (A) and CDC Cowboy (F)

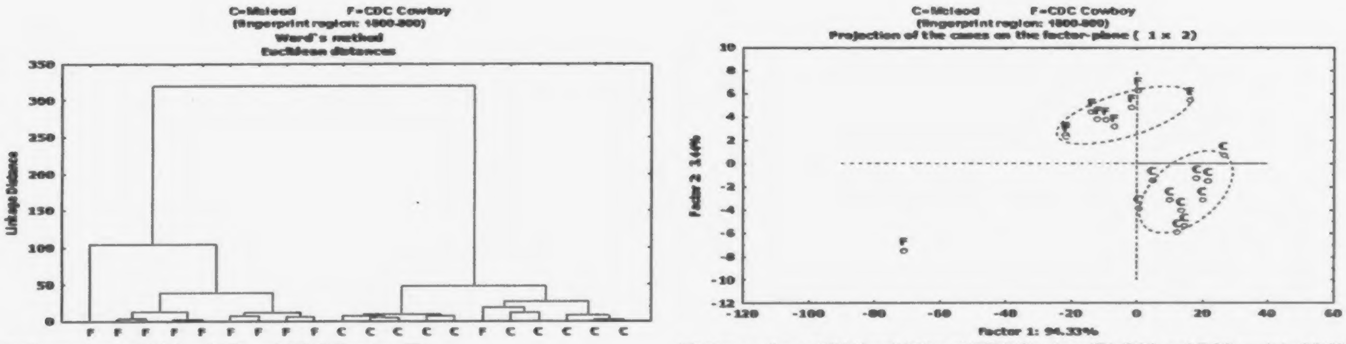
(1) Comparison of AC Metcalfe (A) and CDC Cowboy (F): PC1 and PC2 explain 95.29 and 3.44% of the variances, respectively.



(2) Comparison of CDC Dolly (B) and CDC Cowboy (F)

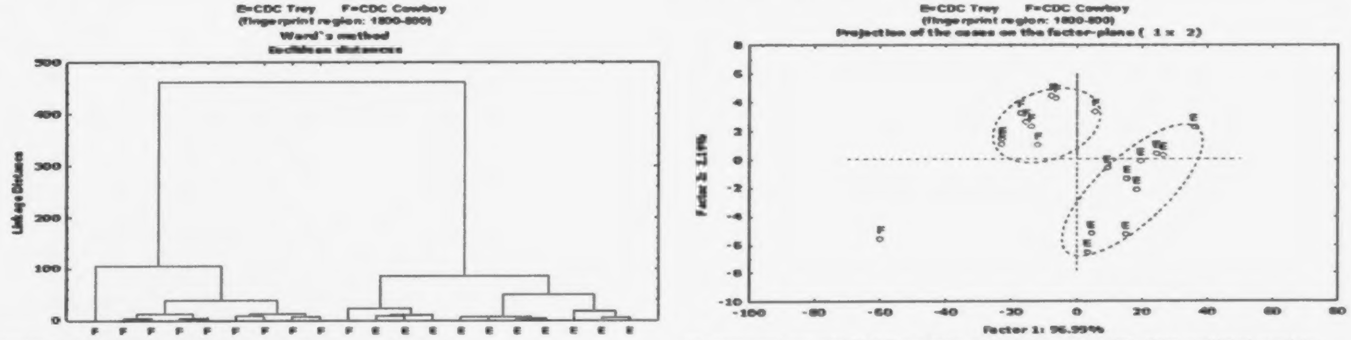
(2) Comparison of CDC Dolly (B) and CDC Cowboy (F): PC1 and PC2 explain 92.08 and 4.58% of the variances, respectively.

Figure A1. Multivariate spectral analyses of barley structures in the whole seed using DRIFT: comparison of CDC Cowboy (F) with other four barley varieties AC Metcalfe (A), CDC Dolly (B), McLeod (C) and CDC Trey (E) I: cluster analysis (1) Select spectral region: finger print region: ~1800 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).



(3) Comparison of McLeod (C) and CDC Cowboy (F)

(3) Comparison of McLeod (C) and CDC Cowboy (F): PC1 and PC2 explain 94.33 and 3.44% of the variances, respectively.



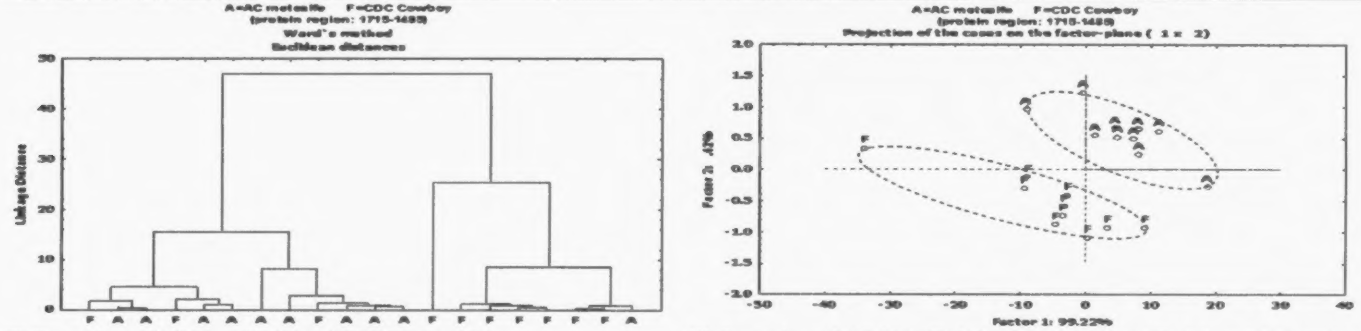
(4) Comparison of CDC Trey (E) and CDC Cowboy (F)

(4) Comparison of CDC Trey (E) and CDC Cowboy (F): PC1 and PC2 explain 96.99 and 2.19% of the variances, respectively.

Figure A1. (continued) Multivariate spectral analyses of barley structures in the whole seed using DRIFT: comparison of CDC Cowboy (F) with other four barley varieties AC Metcalfe (A), CDC Dolly (B), McLeod (C) and CDC Trey (E) I: cluster analysis (1) Select spectral region: finger print region: ~1800 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

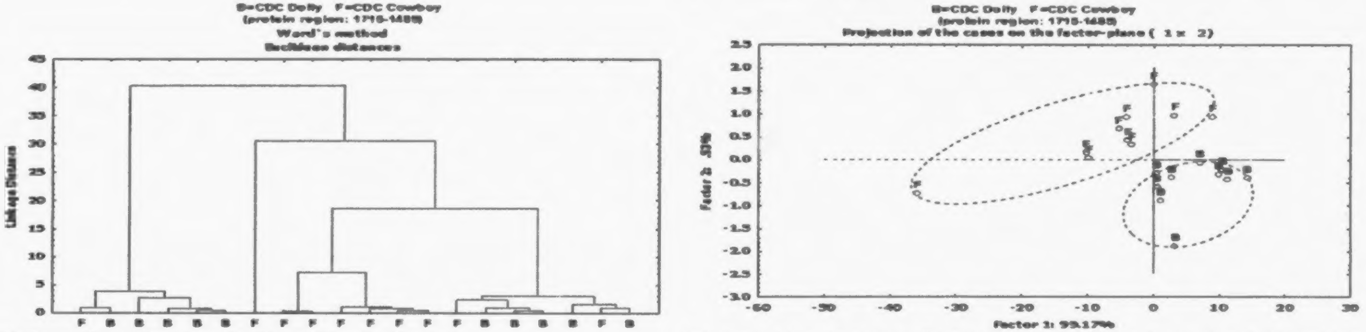
I: Cluster Analysis (CLA): protein region 1715-1485 cm^{-1}

II: Principal component analysis (PCA): protein region 1715-1485 cm^{-1}



(1) Comparison of AC Metcalfe (A) and CDC Cowboy (F)

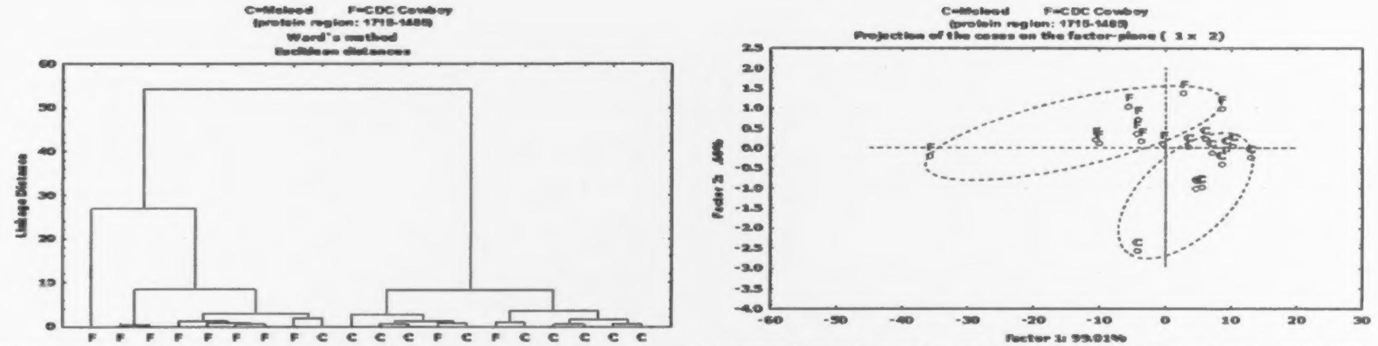
(1) Comparison of AC Metcalfe (A) and CDC Cowboy (F): PC1 and PC2 explain 99.22 and 0.42% of the variances, respectively.



(2) Comparison of CDC Dolly (B) and CDC Cowboy (F)

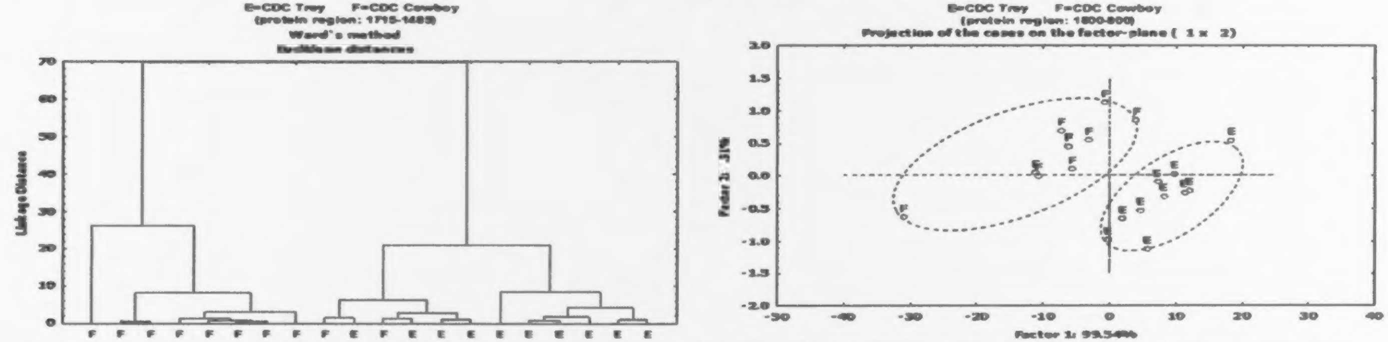
(2) Comparison of CDC Dolly (B) and CDC Cowboy (F): PC1 and PC2 explain 99.17 and 0.53% of the variances, respectively.

Figure A2. Multivariate spectral analyses of barley structures in the whole seed using DRIFT: comparison of CDC Cowboy (F) with other four barley varieties AC Metcalfe (A), CDC Dolly (B), McLeod (C) and CDC Trey (E) I: (1) Select spectral region: amide I and II region: ~1715 to 1485 cm-1; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).



(3) Comparison of McLeod (C) and CDC Cowboy (F)

(3) Comparison of McLeod (C) and CDC Cowboy (F): PC1 and PC2 explain 99.01 and 0.60% of the variances, respectively.

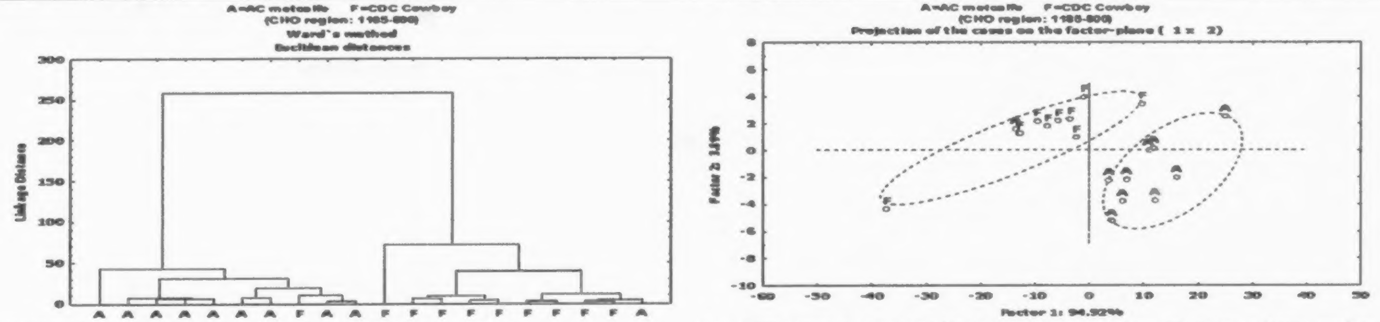


(4) Comparison of CDC Trey (E) and CDC Cowboy (F)

(4) Comparison of CDC Trey (E) and CDC Cowboy (F): PC1 and PC2 explain 99.54 and 0.31% of the variances, respectively.

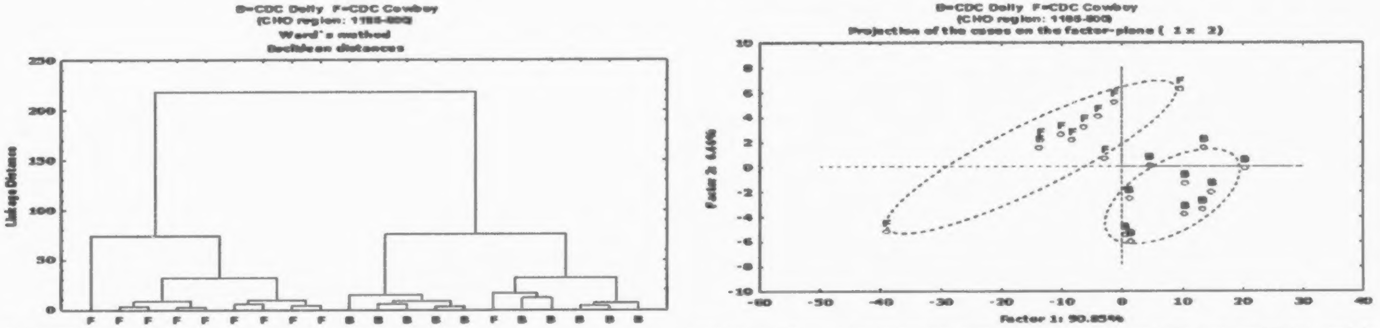
Figure A2. (continued) Multivariate spectral analyses of barley structures in the whole seed using DRIFT: comparison of CDC Cowboy (F) with other four barley varieties AC Metcalfe (A), CDC Dolly (B), McLeod (C) and CDC Trey (E) I: (1) Select spectral region: amide I and II region: ~1715 to 1485 cm-1; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

I: Cluster Analysis (CLA): CHO region 1185-800 cm^{-1} II: Principal component analysis (PCA): CHO region 1185-800 cm^{-1}



(1) Comparison of AC Metcalfe (A) and CDC Cowboy (F)

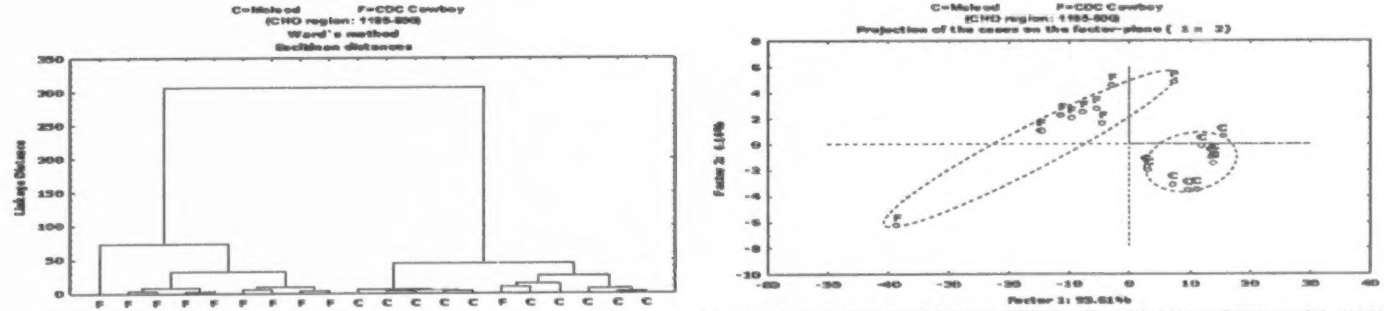
(1) Comparison of AC Metcalfe (A) and CDC Cowboy (F): PC1 and PC2 explain 94.92 and 3.89% of the variances, respectively.



(2) Comparison of CDC Dolly (B) and CDC Cowboy (F)

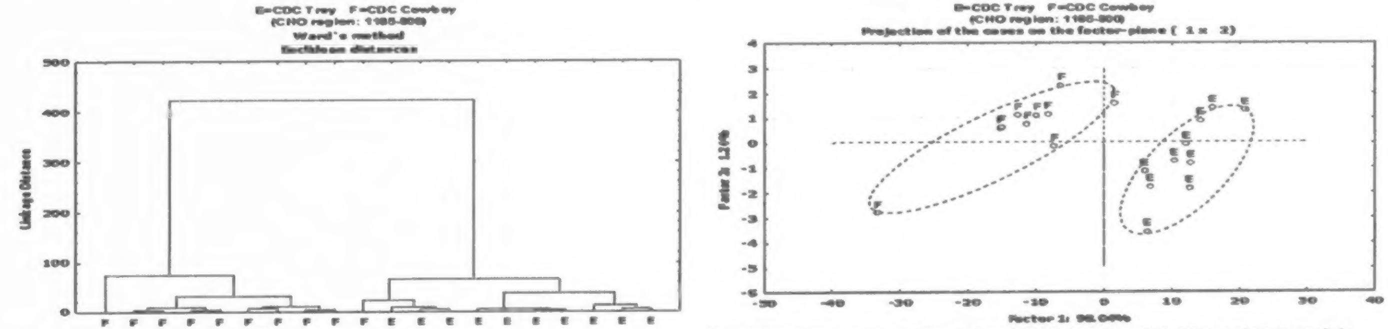
(2) Comparison of CDC Dolly (B) and CDC Cowboy (F): PC1 and PC2 explain 90.85 and 6.46% of the variances, respectively.

Figure A3. Multivariate spectral analyses of barley structures in the whole seed using DRIFT: comparison of CDC Cowboy (F) with other four barley varieties AC Metcalfe (A), CDC Dolly (B), McLeod (C) and CDC Trey (E) I: cluster analysis (1) Select spectral region: carbohydrate molecular region: 1185 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).



(3) Comparison of McLeod (C) and CDC Cowboy (F)

(3) Comparison of McLeod (C) and CDC Cowboy (F): PC1 and PC2 explain 93.61 and 4.14% of the variances, respectively.



(4) Comparison of CDC Trey (E) and CDC Cowboy (F)

(4) Comparison of CDC Trey (E) and CDC Cowboy (F): PC1 and PC2 explain 98.04 and 1.24% of the variances, respectively.

Figure A3. (continued) Multivariate spectral analyses of barley structures in the whole seed using DRIFT: comparison of CDC Cowboy (F) with other four barley varieties AC Metcalfe (A), CDC Dolly (B), McLeod (C) and CDC Trey (E) I: cluster analysis (1) Select spectral region: carbohydrate molecular region: 1185 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

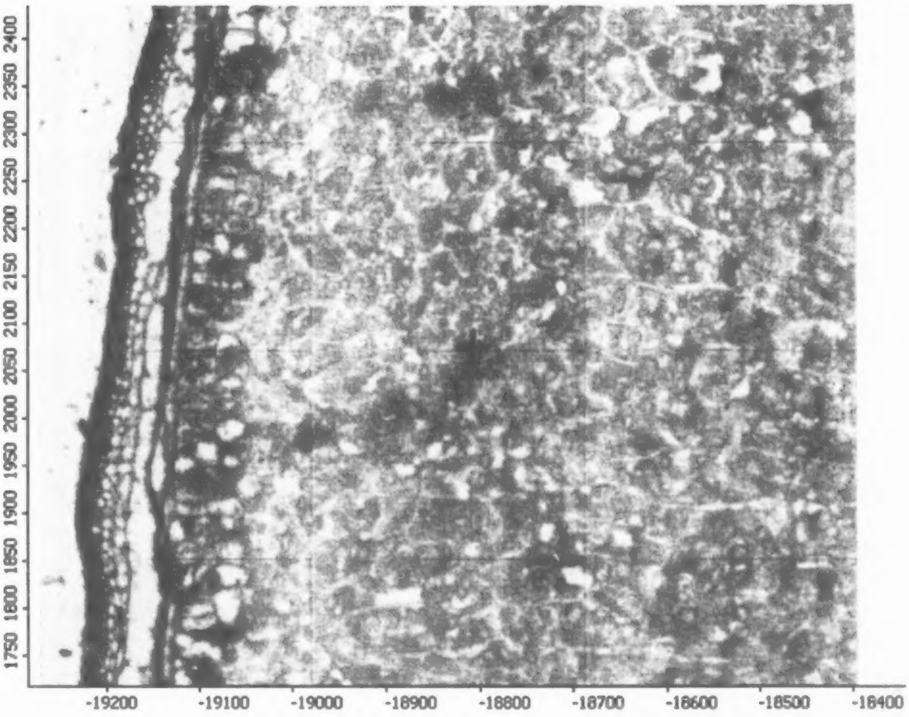

2400
2350
2300
2250
2200
2150
2100
2050
2000
1950
1900
1850
1800
1750
-19200
-19100
-19000
-18900
-18800
-18700
-18600
-18500
-18400

Figure A4. A photomicrograph of barley (cv. McLeod) section produced using ultra-spatial resolution synchrotron-based FTIR microspectroscopy at NSLS U2B station.

Table A.1 Trend of the comparison results of particle size, chemical file, *in situ* rumen degradation kinetics of dry matter (DM), crude protein (CP), starch (ST), and DRIFT and Synchrotron-based FTIR spectroscopic data among six barley varieties.

	AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy
			Particle size			
Mean (mm)	L^z	H^z		H		
Median (mm)	L	H		H		
			In situ rumen degradation kinetics of DM			
S				L	H	
D		L			H	
U		H			L	
Kd	H	L	L		H	
EDDM%	H			L	H	
			In situ rumen degradation kinetics of CP			
CP%	H				L	H
S	H				L	
D		L			H	
U		H	H		L	L
Kd	H		H	L	H	
EDCP%	H	L		L	H	
			In situ rumen degradation kinetics of ST			
ST%	H	H	L			
Kd	H	L		L	H	
EDST%	H	L		L	H	
	AC Metcalfe	CDC Dolly	McLeod	CDC Helgason	CDC Trey	CDC Cowboy
			DRIFT data			
Hull sample						
Lignin area	H	L		H		H
Cellulosic area	L	L		H		H
Total CHO area		L		H	L	
Whole seed sample						
Amide I area		L	L	L		H
Cellulosic area	H			L	L	H
Total CHO area				L		H
			SFTIR data			
Amide I area	L		H	L		
Amide II area	L		H	L		H
Total CHO area		H	L	H		
Cellulosic area	H	H	L	H		
CHO 1st area		H	L	H		
CHO2nd area		H	L	H		
CHO3th (starch)		H	L	H		

[z] H= numerically high value; L= numerically low value.

Highly Qualified Personal Training from This Project:

- MSc Graduate student: Gali (L.) Du. Thesis title: "Hull, Ferulic Acid, Paracoumaric Acid Content and Particle Size Characteristics of Various Barley Varieties in Relation to Nutrient Availability in Ruminants". The thesis was successful defenced on Dec 22, 2008

- MSc Graduate student: Chris (N.) Liu. Thesis title: "Ruminal Nutrient Availability and Inherent Structural Features of Six Barley Varieties Using in Situ Technique and Mid-IR Spectroscopy". The thesis was successful defenced on Jan 19, 2010

The following the detailed Table of Content of graduate student thesis

Graduate student Thesis One: Table of Contents

TABLE OF CONTENTS

PERMISSION TO USE STATEMENT ....
ABSTRACT ....
ACKNOWLEDGEMENTS ....
TABLE OF CONTENTS ....
LIST OF TABLES ....
LIST OF FIGURES ....
LIST OF ABBREVIATIONS ....
1. INTRODUCTION ....
2. LITERATURE REVIEW ....
2.1 Basic Information on Barley and Cattle
2.1.1 Barley
2.1.1.1 Barley Production in Canada
2.1.1.2 Barley Variety
2.1.1.3 Malting Barley and Feed Barley
2.1.1.4 Two-row and Six-row Barley
2.1.1.5 Hulled and Hull-less Barley
2.1.2 Specific Digestion Behavior in Ruminants
2.2 Physical and Chemical Profiles of Barley Grain
2.2.1 Barley Hull
2.2.1.1 The Composite Structure of the Hull
2.2.1.2 The Chemical Constituents in the Hull
2.2.1.3 Factors Influencing Barley Hull Content
2.2.2 Dehulled Barley Kernel
2.2.2.1 Pericarp
2.2.2.2 Testa
2.2.2.3 Aleurone layer
2.2.2.4 Starchy Endosperm
2.2.2.5 Embryo
2.3 Factors Affecting the Digestibility of Barley Grain in Ruminants
2.3.1 Animal Eating Behaviors and Feeding Methods
2.3.1.1 Importance of the Particle Size of Barley Grain after Coarse Dry-rolling
2.3.1.2 Factors Affecting the Particle Size of Barley Grain after Coarse Dry-rolling
2.3.2 Fermentation Activities of Rumen Microorganisms

2.3.3 Physical and Chemical Effects on the Digestibility of Barley Grain
  2.3.3.1 Fiber in Barley Grain
    2.3.3.1.1 Acid Detergent Fiber and Neutral Detergent Fiber
    2.3.3.1.2 Lignin or Acid Detergent Lignin
  2.3.3.2 Hydroxycinnamic Acids in Barley Grain
    2.3.3.2.1 Functions of Ferulic Acid and ρ-Coumaric Acid in Plants
    2.3.3.2.2 Biosynthesis of FA, PCA and Formation of Bound FA
    2.3.3.2.3 Ferulic Acid and ρ-Coumaric Acid in Barley
      2.3.3.2.3.1 ρ-Coumaric Acid
      2.3.3.2.3.2 Monomer of Ferulic Acid
      2.3.3.2.3.3 Dimers of Ferulic Acid
      2.3.3.2.3.4 Trimers of Ferulic Acid
2.3.3.2.3.5 Other Linkages of Ferulic Acid to Limit Plant Cell Wall Digestibility
2.3.4 How to Improve the Digestibility of Barley Plant Cell Walls (e.g. hull)?
2.4 Analyzing Methods for Barley Hull, Ferulic acid, ρ-Coumaric Acid, Particle Size and In Situ Rumen Digestion
  2.4.1 Analysis of Barley Hull Content
  2.4.2 Quantification Methods for Ferulic Acid and ρ-Coumaric Acid
  2.4.3 Particle Size Analysis of Barley Grain
2.5 Summary of Literature Review, Hypothesis and Research Objectives

3. INVESTIGATION OF CHEMICAL AND PHYSICAL DIFFERENCES AMONG THE VARIOUS BARLEY VARIETIES GROWN IN WESTERN CANADA Error! Bookmark not defined.
3.1 Introduction
3.2 Materials and Methods
  3.2.1 Barley Varieties and Samples
  3.2.2 Determination of Barley Hull Content Using Modified European Brewery Convention (EBC) Method
  3.2.3 Determination of the Content of NDF, ADF and ADL
  3.2.4 Determination of Ferulic Acid and ρ-Coumaric Acid Content
    3.2.4.1 Sample Preparation
    3.2.4.2 HPLC Condition
  3.2.5 Determination of Mean/Median Particle Size of Barley Grain Obtained after Dry-rolling
  3.2.6 Statistical Analysis
3.3 Results and Discussion
  3.3.1 Variety Effect on Barley Hull Content
  3.3.2 Variety Effect on the Content of FA, PCA and PCA/FA in Barley Grain
  3.3.3 NDF, ADF, ADL, Hemicellulose and Cellulose in Barley Grain
  3.3.4 Mean/Median Particle Size of Coarsely Dry-rolled Barley Grain
3.4 Conclusions and Implications

4. IN SITU DEGRADATION CHARACTERISTICS OF HYDROXYCINNAMIC ACIDS AND FIBER OF VARIOUS BALREY VARIETIES..
4.1 Introduction
4.2 Materials and Methods
  4.2.1 Barley Samples and Preparation
  4.2.2 In Situ Rumen Incubation
    4.2.2.1 Animals and Diets
    4.2.2.2 Rumen Incubation
      Trial 1: Rumen Degradability of DM, FA, PCA, NDF, ADF and ADL of Six Barley Varieties at 12 and 24 h Rumen Incubation
      Trial 2: In Situ Rumen Degradation Kinetics of Two Barley Varieties
    4.2.2.3 Rumen Degradation Kinetics

4.2.3 Statistical Analysis
4.3 Results and Discussion
4.3.1 Trial 1: Rumen Degradability of DM, FA, PCA, NDF, ADF and ADL of Six Barley Varieties at 12 and 24 h of Rumen Incubations
4.3.2 Trial 2: In Situ Rumen Degradation Kinetics of Ferulic Acid and ρ-Coumaric Acid in Two Barley Varieties
4.3.2.1 Degradation Characteristics of DM
4.3.2.2 Rumen Degradation Characteristics of FA and PCA
4.4 Conclusions and Implications

5. INVESTIGATION OF RELATIONSHIPS BETWEEN ORIGINAL CONTENT OF HULL, HYDROXYCINNAMIC ACIDS, FIBER, PARTICLE SIZES IN VARIOUS BARLEY GRAIN AND IN SITU RUMEN DEGRADABILITIES ....

5.1 Introduction
5.2 Material and Methods
5.2.1 Barley Samples
5.2.2 Chemical Analysis
5.2.3 Statistical Analysis
5.3 Results and Discussion
5.3.1 Correlation Analysis between Original Content of Hull, FA, PCA, Fiber, Particle Sizes and Rumen Indigestible Residues at 12 and 24 h of Rumen Incubation
5.3.1.1 The Effects of Barley Hull
5.3.1.2 The Effects of FA and PCA, Ratio of PCA/FA and total of FA +PCA
5.3.1.3 The Effects of NDF, ADF, ADL, Hemicellulose and Cellulose
5.3.1.4 The Effects of Mean/Median Grain Particle Size
5.3.2 Multi-regression Analysis between Hull, FA, PCA Content, Mean/ median Particle Size and Rumen Indigestible Residues at 12 and 24 h of Rumen Incubation
5.4 Conclusions and Implications

6. GENERAL CONCLUSIONS ....

LITERATURE CITED..

APPENDICES..

LIST OF TABLES

Table

Table 2.1. Chemical composition of barley hull. ....
Table 3.1. Barley samples used in the project..
Table 3.2. Aperture sizes for test sieves ....
Table 3.3. Variation of variety effect on hull content in six barley varieties.......................................
Table 3.4. Variation of variety effect on the content of FA, PCA and ratio of PCA/FA of whole barley grain in six barley varieties ....
Table 3.5. Variation of variety effect on the content of ADF, NDF, ADL, cellulose and hemicellulose in six barley varieties ....
Table 3.6. Variation of variety effect on mean/median particle sizes of coarsely dry-rolled barley predicted by Pond's equation with 0 mm = 100% in six barley varieties ....
Table 4.1. Rumen degradability of DM, FA, PCA, NDF, ADF and ADL of six barley varieties at 12 and 24 h of rumen incubations..
Table 4.2. In situ degradation kinetics of dry matter in CDC Dolly and McLeod..............................
Table 4.3. In situ rumen degradation kinetics of ferulic acid in CDC Dolly and McLeod ....

Table 4.4. In situ rumen degradation kinetics of para-coumaric acid in CDC Dolly and McLeod.....
Table 5.1. Correlation analysis for all parameters (original content of barley hull, FA, PCA, ration, total, NDF, ADF, ADL, hemicellulose, cellulose, mean/ median particle size, and rumen in situ indigestible residues of DM, NDF, ADF, ADL, FA and PCA at 12 and 24 h of rumen incubation) ....

Table 5.2. Multi-Regression analysis to find most important variables to predict rumen degradability using physiochemical characteristics: hull, FA, PCA, ratio, and mean/median particle size with tested multi-regression model as follows: Model: Y (degradability) = Hull + FA+ PCA + PCA/FA ratio + Mean + Median ....

Table A1. Comparison of two methods for determination of barley hull content..........

Table A2. Comparison of the parameters of RSS and R^2, as well as mean/median particle size predicted from Fisher's equation and Pond's equation (with/ without 0 mm = 100%) for coarsely dry-rolled barley grain ....

Table A3. Standard dairy concentrate ....
Table A4. Fresh cow concentrate..........
Table A5. Nylon bags arrangement for 0 - 72 h of rumen incubation..........
Table A6. Detailed 'gradual in/all out' rumen incubation schedule..........

LIST OF FIGURES

Table

Figure 2.1. Diagram of longitudinal section of a barley grain. ....

Figure 2.2. Micro-structure of barley hull and related tissues..........

Figure 2.3. Chemical Structures of Ferulic acid and ρ-Coumaric acid..........

Figure 2.4. Pathway of hydroxycinnamic acids and lignin biosynthesis. ....

Figure 2.5. Cross-linkages between lignin and polysaccharides in plant cell walls. ....

Figure 4.1. Dry matter residue of CDC Dolly and McLeod at various rumen incubations..........

Figure 4.2. Ferulic acid disappearance of CDC Dolly and Mcleod at various rumen incubations

Figure 4.3. para-Coumaric acid disappearance of CDC Dolly and Mcleod at various rumen incubations

Figure A1. HPLC chromatogram at 305 nm of PCA and FA in barley grain extracted after alkaline hydrolysis..........

Graduate student Thesis Two: Table of Contents

TABLE OF CONTENTS

PERMISSION TO USE STATEMENT ....
ABSTRACT..........
ACKNOWLEDGMENTS..........
TABLE OF CONTENTS..........
LIST OF TABLES..........
LIST OF FIGURES ....
LIST OF APPENDIX..........
LIST OF ABBREVIATIONS..........

1. LITERATURE REVIEW..
- 1.1. Barley in ruminant nutrition
  - 1.1.1. Background
    - 1.1.1.1. History, production and seed structure
    - 1.1.1.2. Uses and economic value
  - 1.1.2. Varieties
    - 1.1.2.1. Barley varieties for malting purpose
    - 1.1.1.2. Barley varieties for feed purpose
  - 1.1.3. Digestion in rumen
    - 1.1.3.1. Nutritional benefits for ruminants
    - 1.1.3.2. Structural characteristic of barley on ruminal nutrient availability
    - 1.1.3.3. Processed barley
    - 1.1.3.4. Degradation behaviours of barley
    - 1.1.3.5. Rumen disorders caused by fast degradation
- 1.2. Normal feed evaluation methods
  - 1.2.1. In vitro and in vivo techniques and nutrient availability and supply modeling
  - 1.2.2. Disadvantages of traditional chemical analysis
- 1.3. Mid-IR spectroscopy application in feed science
  - 1.3.1. Infrared spectroscopy principles
    - 1.3.1.1. Basic knowledge
    - 1.3.1.2. Spectral band assignments and characteristics
  - 1.3.2. Spectral analysis
    - 1.3.2.1. Univariate spectral analysis
    - 1.3.2.2. Multivariate spectral analysis
  - 1.3.3. Fourier transform infrared spectroscopy
    - 1.3.3.1. Diffuse reflectance Fourier transform infrared spectroscopy
    - 1.3.3.2. Synchrotron-based FTIR microspectroscopy
      - 1.3.3.2.1. Synchrotron

      - 1.3.3.2.2. Advantage of synchrotron light

  - 1.3.4. Biological application of Mid-IR spectroscopy and recent progress in feed science
    - 1.3.4.1. Application of FTIR techniques
    - 1.3.4.2. Application of FTIR microspectroscopy
    - 1.3.4.3. Application of synchrotron-based FTIR microspectroscopy (SFTIRM)
- 1.4. Conclusions and research hypothesis

2. PARTICLE SIZE, CHEMICAL PROFILE AND *IN SITU* RUMEN DEGRADATION KINETICS OF DRY MATTER, CRUDE PROTEIN AND STARCH AMONG SIX BARLEY VARIETIES: EVALUATION AND CORRELATION
- 2.1. Introduction
- 2.2. Materials and methods
  - 2.2.1. Barley varieties and processing method
  - 2.2.2. Particle size analysis
  - 2.2.3. Animal work
    - 2.2.3.1. Animals and diets
    - 2.2.3.2. Rumen incubation
  - 2.2.4. Chemical analysis
  - 2.2.5. Rumen degradation kinetics
  - 2.2.6. Statistical analysis
- 2.3. Results and discussion
  - 2.3.1. Comparison of mean and median particle size of coarsely dry-rolled barley grains among six barley varieties

    - 2.3.2. Evaluation of chemical profile and *in situ* rumen degradation kinetics of dry matter (DM), crude protein (CP) and starch (ST) among six barley varieties
    - 2.3.3. Correlation between mean and median particle sizes and degradation rate and extent of dry matter (DM), crude protein (CP) and starch (ST) among barley varieties
  - 2.4. Conclusions 66

3. USING DIFFUSE REFLECTANCE FOURIER TRANSFORM INFARED SPECTROSCOPY (DRIFT) AND SYNCHROTRON-BASED FTIR MICROSPECTROSCOPY (SFTIRM) TO CHARACTERIZE SEED INHERENT STRUCTURE AMONG SIX BARLEY VARIETIES

- 3.1. Introduction
- 3.2. Materials and methods
  - 3.2.1. Barley varieties and growth condition
  - 3.2.2. Sample preparation for DRIFT spectroscopy
  - 3.2.3. DRIFT Spectroscopy
  - 3.2.4. DRIFT data collection and processing
  - 3.2.5. Sample preparation for SFTIR microspectroscopy
  - 3.2.6. SFTIR microspectroscopy
  - 3.2.7. SFTIRM data collection and processing
  - 3.2.8. Multivariate spectral analysis
  - 3.2.9. Statistical analysis
- 3.3. Results and discussion
  - 3.3.1. Using DRIFT spectroscopy to characterize and compare structure and chemical make-up of hull samples from six barley varieties
  - 3.3.2. Using DRIFT spectroscopy to characterize and compare structure-chemical make-up of whole seed samples from the six barley varieties
  - 3.3.3. Using Synchrotron-based FTIR microspectroscopy (SFTIRM) to characterize and compare structural and chemical make-up of seed endosperm tissue among six barley varieties
  - 3.3.4. Correlations between mean and median particle sizes, *in situ* degradation features of DM, CP, and starch and Mid-IR spectral characteristics among barley varieties
  - 3.3.5. Using Mid-IR spectroscopy with multivariate molecular spectral analysis to discriminate and classify molecular structure difference of barley seed: Comparison among barley varieties
- 3.4. Conclusions

4. GENERAL DISCUSSION AND CONCLUSIONS..

LIST OF REFERENCES...

APPENDIX: SUPPLEMENTARY MATERIAL..

LIST OF TABLES

Table

Table 1.1 Chemical composition of Harrington and Valier barley
Table 1.2 Brief summary of typical IR spectral bands of biological compounds.
Table 2.1 Particle size comparison of six barley varieties
Table 2.2 Comparison of six barley varieties: chemical profile and *in situ* rumen degradation kinetics of dry matter (DM).
Table 2.3 Chemical profile and *in situ* rumen degradation kinetics of crude protein (CP): Comparison of six barley varieties.
Table 2.4 Chemical profile and *in situ* rumen degradation kinetics of starch (ST): Comparison of six barley varieties.
Table 2.5 Pearson correlations between *in situ* rumen degradation rate and extent of dry matter (DM), crude protein (CP) and starch (ST) from six barley varieties and mean and median of particle sizes.
Table 2.6 Regression analysis to predict rumen degradability using median particle size (x) with regression model

Table 3.1 The structural characteristics of aromatic lignin, cellulosic compound and total carbohydrate and their ratios in the barley hull, revealed using DRIFT spectroscopy: Comparison of six barley varieties (n = 10 samples per each type of barley hull).

Table 3.2 The structural characteristics of protein amide I and II, carbohydrates, structural carbohydrate (cellulosic compound) and their ratios in the whole barley seed, revealed using DRIFT spectroscopy: Comparison of six barley varieties (n = 10 samples per each type of whole barley seed).

Table 3.3 The structural characteristics of protein amides I and II in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy: Comparison of six barley varieties.

Table 3.4 The structural characteristics of carbohydrates, and structural carbohydrate (cellulosic compounds) in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy: Comparison of six barley varieties.

Table 3.5 The structural characteristics of the ratios of protein amide I and II, and structural (cellulosic compounds) and non-structural (NSC, starch) carbohydrates (CHO) in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy: Comparison of six barley varieties.

Table 3.6. Pearson correlation results between structural characteristics from hull sample and *in situ* rumen degradation rate and extent of six barley varieties. (The structural characteristics include aromatic lignin, cellulosic compound and total carbohydrate and their ratios in the barley hull, revealed using DRIFT spectroscopy).

Table 3.7. Pearson correlation results between structural characteristics from whole seed sample and *in situ* rumen degradation rate and extent of six barley varieties. [The structural characteristics include protein amide I and II, carbohydrates, structural carbohydrate (cellulosic compound) and their ratios in the whole barley seed, revealed using DRIFT spectroscopy.]

Table 3.8. Pearson correlation results between structural characteristics from seed endosperm tissue and *in situ* rumen degradation rate and extent of six barley varieties. (The structural characteristics include protein amides I and II in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy.)

Table 3.9. Pearson correlation results between structural characteristics from seed endosperm tissue and *in situ* rumen degradation rate and extent of six barley varieties. [The structural characteristics include carbohydrates and structural carbohydrate (cellulosic compounds) in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy.]

Table 3.10. Pearson correlation results between structural characteristics ratios from seed endosperm tissue and *in situ* rumen degradation rate and extent of six barley varieties. [The structural characteristics include protein amide I and II, and structural (cellulosic compounds) and non-structural (NSC, starch) carbohydrates (CHO) in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy.]

Table 3.11. Pearson correlation results between structural characteristics from seed endosperm tissue and *in situ* rumen degradation characteristics of six barley varieties. [The structural characteristics include structural carbohydrate (cellulosic compounds) and amide I: cellulosic compound ratio in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy]

LIST OF FIGURES

FIGURES

Figure 2.1 Scanning electron micrograph of mature starchy endosperm cells of wheat. (a) Tissue from the central starchy endosperm of cv Bouquet showing small (SB) and large (SA) starch granules embedded in a protein matrix (P). (b) Enlargement to show the protein matrix (P) in starchy endosperm tissue of cv Avalon.

Figure 3.1 Typical FTIR spectrum of barley hull: (a) whole mid-IR region: ca. 4000-800 cm^{-1}; (b) Fingerprint region: ca. 1800-800 cm^{-1}; (c) Aromatic lignin peak area: ca. 1510 cm^{-1}; (d) Total carbohydrate peak area region: ca. 1192-932 cm^{-1}; (e) Cellulosic compound peak area: ca 1245 cm^{-1}.

Figure 3.2 Typical FTIR spectrum of whole barley seed: (a) Fingerprint region: ca. 1800-800 cm^{-1}; (b) Total carbohydrate peak area region: ca. 1188-955 cm^{-1} (c,d) Amide I peak area and height: ca. 1650 cm^{-1}; (e,f) Cellulosic compound peak area and height: ca 1245 cm^{-1}.

Figure 3.3 Typical synchrotron-based FTIR spectrum of endosperm tissue within a cellular dimension: (a) Whole mid-IR region: ca. 4000-800 cm^{-1}; (b) Fingerprint region: ca. 1800-800 cm^{-1}; (c,d) Amide I peak area and height: ca. 1650 cm^{-1}; (e,f) Amide II peak area and height: ca. 1650 cm^{-1}; (e,f) Amide II peak area and height: ca. 1550 cm^{-1}; (g, h) Cellulosic material peak area and height: ca 1240 cm^{-1}

Figure 3.4 Typical synchrotron-based FTIR spectrum of endosperm tissue within a cellular dimension: (a) Total carbohydrate peak area region: ca. 1184-950 cm-1; (b,c) Carbohydrate 1st component peak area and height: ca. 1150 cm-1; (d,e) Carbohydrate 2nd component peak area and height: ca. 1080 cm^{-1}; (f,g) carbohydrate 3rd component peak area and height: ca. 1025 cm^{-1}.

Figure 3.5 Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgson (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral region: fingerprint region: ca. 1800 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

Figure 3.6 Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgason (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral region: amides I and II region: ca. 1715 to 1485 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

Figure 3.7 Multivariate spectral analyses of barley structures in the whole seed: comparison of CDC Helgason (D) with other five barley varieties (AC Metcalfe (A), CDC Dolly (B), McLeod (C), CDC Trey (E) and CDC Cowboy (F) I: cluster analysis (1) Select spectral region: carbohydrate region: ca. 1185 to 800 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

Technology Transfer, Scientific Publications and Presentations/ Seminars

(*Corresponding author and graduate student supervisor)

1. L. Du, Peiqiang Yu*, B.G. Rossnagel, D.A. Christensen, J.J. McKinnon. 2009. Physicochemical Characteristics, Hydroxycinnamic Acids (Ferulic acid, ρ-Coumaric Acid) and Their Ratio and In situ Biodegradability: Comparison Genotypic Differences among Six Barley Varieties. **Journal of Agricultural and Food Chemistry**. 57 (11): 4777–4783 (*corresponding authors)

2. L. Du and Peiqiang Yu*. 2009. Effect of Barley Variety and Growth Year on Ferulic and Para-Coumaric Acids and Their Ratios in the Seed and Hull. **Cereal Research Communications**. In Press (139/2009.R2) (Fully Accepted on 2Mar10).

3. L. Du and Peiqiang Yu*. 2009. Relationship of Physicochemical Characteristics and Hydroxycinnamic Acids Profile (Ferulic acid, p-Coumaric Acid, Their Ratio) of CDC Barley Varieties and Nutrient Availability in Ruminants. **Journal of Cereal Science (Elsevier)**. In Review (JCS10-17)

4. N. Liu and Peiqiang Yu*. 2010. Characterize Microchemical Structure of Seed Endosperm within a Cellular Dimension among Six Barley Varieties with Distinct Degradation Kinetics, Using Ultraspatially Resolved Synchrotron-Based Infrared Microspectroscopy. **Journal of Agricultural and Food Chemistry**. In Press (jf-2010-01233n) (Fully Accepted on 16May10).

5. N. Liu and Peiqiang Yu*. 2010. Using DRIFT Molecular Spectroscopy with Uni- and Multi-variate Molecular Spectral Techniques to Detect Plant Protein Molecular Structure Difference among Different Genotypes of Barley. **Journal of Agricultural and Food Chemistry.** In Press (jf-2010-003545 R1) (Fully Accepted on 19Apr10)

6. N. Liu and Peiqiang Yu*. 2010. Correlation between Median and Mean Irregular Particle Sizes and Degradation Kinetics in Barley Genotypes. **Journal of Animal Physiology and Animal Nutrition.** In Review (JAPAN-Jan-10-015.R1) (Fully Accepted on 29Mar10).

7. N. Liu and Peiqiang Yu*. 2010. Molecular Clustering, Interrelationships and Carbohydrate Conformation in Hull and Seeds among Barley Cultivars: Detect Genotypic Difference. **Journal of Agricultural and Food Chemistry**. In review (jf-2010-01563q).

8. N. Liu, J.J. McKinnon, D.A. Christensen and Peiqiang Yu*, 2010. Characterize Microchemical Structure of Seed Endosperm within a Cellular Dimension among Six Barley Varieties, Using Ultraspatially Resolved Synchrotron-Based Infrared Microspectroscopy. In: **Life Sciences Section, Canadian Light Source (CLS: Synchrotron) Activity Report 2009**, Editor, M. Dalzell.

9. N. Liu, and Peiqiang Yu*, 2009. Using IR Spectroscopy and Chemometrics with Uni-and Multivariate Analyses to Characterize Structure and Biodegradation Kinetics: Comparison of Hull and Seed Inherent Structures between Two Barleys. In: **Life Science Section, Canadian Light Source (CLS: Synchrotron) Activity Report 2008** (Book ISBN 978-0-9783761-2-3), M. Dalzell, Editor, pp 136-137.

10. Peiqiang Yu*, N. Liu, K. Doiron, D. Liu, Z. Niu, C.R. Christenson, D.A. Christensen and J. J. McKinnon, 2008. Structural Conformation and Microlocalization of Digestion-Resistant Biopolymers /Compounds in Feeds at Cellular Dimensions. In: **Life Science Section, Canadian Light Source Activity Report 2007** (Book ISBN 978-0-9783761-1-6), M. Dalzell, Editor, pp 128-129 (* As PI)

11. N. Liu, J.J. McKinnon, B. Rossnagel, D.A. Christensen, Peiqiang Yu* 2010. Using Synchrotron-Based FTIR Microspectroscopy and DRIFT Spectroscopy with Uni- and Multivariate Molecular Spectral Techniques for Barley Structure and Biodegradation Research. **The 6th Canadian Barley Symposium**, Saskatoon, Saskatchewan, Canada, July 25 - 28, 2010 (Invited upcoming conference).

12. N. Liu, P. Yu*. 2010. Using Synchrotron-based FTIR Microspectroscopy (SFTIRM) to Reveal the Differences of Endosperm Structural and Chemical Makeup among Six Barley Varieties. **Soils and Crops 2010**, University of Saskatchewan, Saskatoon, SK, Canada, February 24-25, 2010 (Full paper).

13. Du, L., N. Liu and P. Yu*. 2009 Determinate the Best Model for Computing Mean/Median Particle Sizes of Coarsely Dry-Rolled Barley-A Comparison Study. **Proceedings of 2009 Inaugural ASAS-CAAVM Pacific Rim Conference**, November 8-10, 2009, Beijing China, pp 46.

14. N. Liu, P. Yu 2009. Correlation between mean and median particle size and ruminant nutrient availability in barleys. **Soils and Crops 2009**, Saskatoon, SK, Canada, February 25-26, 2009.

15. N. Liu, P. Yu*, J.J. McKinnon and D.A. Christensen. 2008. Nutrient Availability of Four CDC Barley Varieties for Ruminants in Comparison with Two Normal Varieties. **Canadian Society of Animal Science (CSAS) Annual Conference**, Guleph, ON, August 11-14, 2008 (Abstract RP19)

16. N. Liu, P. Yu*, J.J. McKinnon and D.A. Christensen. 2008. Using Advanced Synchrotron Radiation Based BioAnalytical Technique to Study Inherent Structures of Six Barley Varieties within Cellular and Subcellular Dimensions. **Canadian Society of Animal Science (CSAS) Annual Conference**, Guleph, ON, August 11-14, 2008 (Abstract RP20)

17. N. Liu, J.J. McKinnon, D.A. Christensen, and P. Yu*, 2008. Discrimination and Classification of Six Barley Varieties Using Infrared Spectroscopy with Multivariate Techniques-AHCA and PCA. **The 11th Canadian Light Source Annual Users' Meeting and Associated Workshops**, University of Saskatchewan, Saskatoon, Canada, June 9 to 10.

18. N. Liu, J.J. McKinnon, D.A. Christensen, and P. Yu*, 2008. Using in situ Technique to Estimate Nutrient Availability of Six Barley Varieties for Ruminants. **Soils and Crops Conference-2008**, Saskatoon, SK, Canada. Feb 28-29 (*As supervisor and PI).

19. L. Du and P. Yu.*. 2007. Hydroxycinnamic Acids in Feed in relation to Rumen Biodegradability. In: **Proceedings of the VII International Symposium on the Nutrition of Herbivores (ISNH7)**, Edited by Q.X. Meng, L.P. Ren, Z.J. Cao, China Agricultural University Press, Beijing, China, Sept. 16-21, 2007, pp 574.

20. L. Du, B.G. Rossnagel, J.J.McKinnon, V. Racz, D.A. Christensen and P. Yu*. 2007. Hull, hydroxycinnamic acids contents and particle size distribution of various barley varieties in relation to nutrient availability in ruminant. In: **Proceedings of the Canadian Nutrition Congress 2007** (CNC-2007 was a joint meeting of the Canadian Section of the American Oil Chemists' Society (CAOCS), the Canadian Society of Animal Science (CSAS), and the Canadian Society for Nutritional Sciences (CSNS), Winnipeg, Manitoba, Canada, June 18-21, 2007, pp. 26

21. L. Du, B.G. Rossnagel, J.J.McKinnon, V. Racz, D.A. Christensen, P. Yu*. 2007. Effect of barley variety on hull content and compassion among various barley varieties. In: **Proceedings of the Canadian Nutrition Congress 2007**, Winnipeg, Manitoba, Canada, June 18-21, 2007, pp. 59 (*As supervisor).

22. L. Du, B.G. Rossnagel, J.J.McKinnon, V. Racz, D.A. Christensen, P. Yu*. 2007. Effect of barley variety on ferulic acid and para-coumaric acid concentrations and compassion among various barley varieties. In: **Proceedings of the Canadian Nutrition Congress 2007** Winnipeg, Manitoba, Canada, June 18-21, 2007, pp. 59 (*As supervisor).

23. N. Liu, and P. Yu*, J.J. McKinnon, D.A. Christensen. 2007. In-Depth Study the Relationship between Inherent Structural Features of Barley Varieties and Nutrient Degradability and Availability. In: **Proceedings of the 10th Canadian Light Source Annual Users' Meeting and Associated Workshops**, University of Saskatchewan, Saskatoon, Canada, June 16 to 17, 2007, pp. 83 (*As supervisor).

24. L. Du, B.R. Rossnagel, J. J. McKinnon, V. Racz, D.A. Christensen, and P. Yu*. 2007. Effect of barley variety on ferulic acid and para-coumaric acid concentrations and comparison among various barley varieties. In: **Proceedings of the 2007 Soils and Crops Conference,** University of Saskatchewan, Saskatoon, SK, Canada. March 1 & 2, 2007 (*As supervisor, Principle Investigator).

25. L. Du, B.R. Rossnagel, J. J. McKinnon, V. Racz, D.A. Christensen, and P. Yu*. 2007. Hull content of seventeen barley varieties and breeding lines. In: **Proceedings of the 2007 Soils and Crops Conference,** University of Saskatchewan, Saskatoon, SK, Canada. March 1 and 2, 2007 (*As supervisor).

26. Du, L. B. Rossnagel, J. J. McKinnon, V. Racz, D.A. Christensen, and P. Yu*. 2007. Hull, hydroxycinnamic acids contents and mean/median particle sizes of barley varieties and breeding lines. **2007-Mini-Conference,** Department of Animal and Poultry Science, University of Saskatchewan, March 9, 2007.

27. Du Liqin, B.G. Rossnagel, J.J. McKinnon, V. Racz, D.A. Christensen, P. Yu*. 2007. Ferulic acid and para-coumaric acid concentration of barley varieties and breeding lines. **Canadian Seed Growers' Association Annual Conference,** University of Saskatchewan, Saskatoon, SK, Canada, July 11-14, 2007 (*As principal investigator).